ANNUAL REPORT
OF
PROGRAM ACTIVITIES
NATIONAL CANCER INSTITUTE
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PART II-B

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
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ANNUAL REPORT

OF

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U.S. NATIONAL CANCER INSTITUTE

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Part II-B

Division of Cancer Biology and Diagnosis

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DIVISION OF CANCER BIOLOGY AND DIAGNOSIS

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Description

The Tumor Biology Program supports a very broad spectrum of biological and biochemical research in the area of cancer biology. The major long-term objective of the program is to develop fundamental information that will help us understand key factors responsible for the expression and control of the malignant phenotype. Of particular interest are those processes that contribute to loss of growth control and lead to penetration and invasion of surrounding tissues. Nearly every aspect of cancer cells is being examined in relation to normal cell behavior with the hope that identification of unique properties associated with neoplastic progression ultimately will lead to improved treatment, therapy and diagnosis of cancer. The basic information developed in this program area is of fundamental importance to nearly every other program area of the National Cancer Institute.

Accomplishments

Several areas of the Tumor Biology Program appear to be developing very successfully and potentially are on the verge of revealing some important facts about the abnormal behavior of cancer cells. Although much of the research supported by this program has continued to develop information that distinguishes biochemical differences between neoplastic cells and and normal cells, the importance of these observations in terms of whether they contribute primarily or secondarily to the maintenance of neoplastic properties remains unknown. In a few cases, however, some of these biochemical differences are being translated into functional differences that are beginning to explain some of the aberrant behavior of cancer cells. Once the neoplastic state is established, regardless of the causative factors involved, it becomes important only to know how processes such as growth and invasion can be controlled.

For many years scientists have suspected that differences in the cell surface membranes of normal and cancer cells are important, but it has not been possible to use these differences to explain altered cell behavior. The experimental approach of purifying surface membranes to study differences has proved to be unsuccessful in the past, so many investigators have turned to studying one or a few specific membrane proteins that are known to change as a result of neoplastic transformation. Some of the most exciting and possibly most significant observations have centered around studies of the protein, fibronectin, which has been shown to exist in the extracellular matrix and surface membrane of cultured fibroblasts, as well as many other kinds of cultured cells (Furcht *et al*, 1978a; Birdwell *et al*, 1978; Quaroni *et al*, 1978; Chen *et al*, 1978). Although interest in this glycoprotein was originally stimulated because of its disappearance after viral transformtion of fibroblast cells, it is now well-established that a similar protein is present in many different mammalian and avian species and that purified

fibronectin can induce changes in cell adhesiveness and cell shape, both of which are properties often altered during tumorigenesis. Fibronectin binds to collagen (Enguall et al, 1978; Ruoslahti and Hayman, 1979), a major component of the extracellular matrix (or basement membrane) that provides what is believed to be an essential adhesive that binds cells together. As cells reach a higher density, crowd each other in culture, and make closer contacts, fibronectin appears to become organized into an external filamentous matrix that often appears to be associated with intracellular actin filaments (Heggness et al, 1978; Furcht et al, 1978b; Furcht et al, 1978c; Hynes et al, 1978). More recently, it has been reported that although no strict correlation can be made between tumorigenic potential and loss of fibronectin, an almost perfect correlation can be made between loss of fibronectin and increased metastasis (Chen et al, 1979). This is a very important observation to confirm. Taken together, results obtained so far could be significant because it is possible to speculate that fibronectin serves as one important common denominator in cell processes such as attachment, spreading and alignment, as well as cell movement. One might expect, and often observes, all of these processes to be altered in metastatic cell populations.

Understanding factors that regulate cell shape and cell movement are likely to be critical to a comprehensive understanding of neoplastic cell behavior. A successfully invasive cancer cell must be able to move and change its shape rather easily if it is to migrate through normal tissues and eventually establish new foci for tumor growth. These two properties of cells, shape and movement, appear to be partially dependent upon the organization of cytoskeletal networks, as well as upon their association with the cell surface (Furcht and Wendelschafer-Crabb, 1978). Microtubule-like structures form networks that are likely candidates for maintaining the shape or super-structure of the cell while actin filaments are prime candidates as contractile elements that serve as minimuscles during cell movement (Goldman et al, 1979; Wang and Goldman, 1978). One way in which changes in cell shape and cell movement could be regulated is through control of the subunit assembly of microtubules and cytoplasmic fibers. It has recently been observed that the assembly-disassembly of both microtubules (Marcum et al, 1978; Welsh et al, 1978) and actin-myosin fiber bundles (Yerna et al, 1979a; Yerna et al, 1979b) appear to be regulated by calcuim ion dependent regulator proteins. Future research will have to assess whether these regulator proteins are related and possibly whether they are somehow linked to the function of fibronectin at the cell surface. These kinds of observations emphasize the importance that ubiquitous divalent metal cations, such as Ca (2+) and Mg (2+), may play in cellular control processes (Rubin et al, 1978). In addition, there is a continued indication that control processes within the cell are highly interdependent. Thus, while there may be inherent inaccuracies in making firm conclusions about the contribution of any one process to neoplasia, studying individual control processes is beginning to pay off in terms of understanding the interrelationship of separate control processes and how they may be manipulated to change cell behavior.

A full understanding of regulatory and control processes that change during tumorigenesis may ultimatyely rely heavily on new techniques rapidly developing in the area of mammalian cell genetics. For example, the use of

cell fusion between normal and malignant cell pairs to examine tumor-forming ability in hybrid cells and their descendents is beginning to yield important information about the neoplastic phenotype. In principle, this approach should permit the identification of individual chromosomes or cytoplasmic DNA's and eventually the specific genes associated with the expression and suppression of tumor-forming ability. Recent results indicate that the malignancy of hybrids, as measured by tumorigenicity, and expression of the transformed phenotype, as measured by anchorage dependence and agglutinability, are not always under coordinate control as might be expected (Jha *et al*, 1978; Stanbridge and Wilkinson, 1978). Also, hybrid analysis between nucleated cells and enucleated cells has demonstrated that tumorigenicity is not cytoplasmicly transmitted (Ziegler, 1978; Howell and Sager, 1978). As genetic techniques become perfected, more specific questions can be asked and more easily related to morphological and biochemical experimental approaches such as those already discussed.

Much of the ultimate success of any research supported by the Tumor Biology Program will depend upon further development of experimental model systems that are suitable for both *in vitro* and *in vivo* biochemical and genetic analysis. There is no doubt that the mouse melanoma model for studying metastasis (Nicolson, 1978) is receiving greater attention and eventually will yield substantial information related to the specificity of arrest, survival and growth of secondary metastatic foci. Because one model system may not be representative of the cancer process, additional systems will be needed if a truly comprehensive picture of the invasive and metastatic process is to be developed. Recently, successes have been achieved in developing models of lymphosarcoma and vasoformative sarcoma that can be studied for different metastatic potential and organ-site selectivity (Nicolson *et al*, 1978; Brunson and Nicolson, 1978; Nicolson, 1979). The repeated success of this approach to developing new model systems is extremely significant to future progress in the areas of tumor biology as well as tumor immunology.

FISCAL YEAR 1979

TUMOR BIOLOGY PROGRAM

SUMMARY BY SUB-CATEGORY
(Dollars in Thousands)

	NONCOMPETING		COMPETING		TOTAL	
	NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT
CELL SURFACE	56	$ 4,546	29	$ 2,657	85	$ 7,203
ENZYMES	25	1,965	13	998	38	2,963
PEPTIDE HORMONES	9	721	8	674	17	1,395
STEROIDS	14	1,248	8	649	22	1,897
MEMBRANEOUS ORGANELLES	10	1,518	4	528	14	2,046
RIBOSOMES AND POLYRIBOSOMES	11	1,410	1	65	12	1,475
M-RNA	15	1,350	3	257	18	1,607
T-RNA	5	401	2	184	7	585
DNA	21	2,019	7	431	28	2,450
GROWTH FACTORS	10	905	1	66	11	971
NUCLEUS	20	1,478	4	268	24	1,746
CONTRACTILE ELEMENTS	5	418	2	154	7	572
DEVELOPMENT AND DIFFERENTIATION	35	2,928	18	1,552	53	4,480
CELL GROWTH, CELL DIVISION	22	1,846	11	881	33	2,727
SOMATIC CELL GENETICS	10	1,005	3	354	13	1,359
INHERITANCE OF NEOPLASMS	2	125	1	50	3	175
PLASMIDS, VIRUSES	2	224	3	201	5	425
VO - PROGRAM PROJECTS	9	5,767	1	530	10	6,297
MISCELLANEOUS	4	271	10	746	14	1,017
NOT CLASSFIED	5	326	4	652	9	978
TOTAL TUMOR BIOLOGY	290	30,471	133	11,897	423	42,368

Description:

The goal of the Immunology Program of the National Cancer Institute is to support research which defines as clearly as possible the multitude of interactions which take place between tumors and the immune system, which attempts to modulate these interactions for the benefit of the host and the detriment of the tumor and which applies immunological techniques to assessment of the host-tumor relationship. Research in immunology touches upon questions of the cause, prevention, detection, diagnosis, therapy and biology of cancer. Emphasis is given to fundamental mechanistic studies on the immune response to tumors as these provide the seeds from which the other more applied efforts develop. The following selected examples illustrate both the breadth of the program and some areas which appear to hold special promise.

A. One of the most significant factors in the prognosis of the cancer patient is the formation of metastases - the multiple tumor colonies that result from spread of the primary tumor. Clinically, such disseminated tumors present significant problems in treatment strategy as selectivity of treatment between normal and malignant tissue in multiple sites limits the potential of surgery and radiation therapy. It thus is important to support studies whose goal is to define the effect of metastasis on protective anti-tumor immune responses. A key concept in such studies is that individual tumors show great heterogeneity in their composite cells. Nicolson, et al., (1978) have demonstrated that selective pressures can be used to develop many cell lines from one tumor. Lines differing greatly in their ability to metastasize also differ in many other biological and biochemical characteristics including display of cell surface proteins (Brunson, et al., 1978). In comparing immune reactivity against a primary tumor and a metastatic variant of that tumor, Fogel, et al., (1978) found that cytotoxic lymphocytes, induced by exposure to the primary tumor, killed that tumor well but were not as effective in lysis of the metastatic variant. The converse was true using cytotoxic lymphocytes induced by the variant. This suggests a difference in antigenicity between primary tumor cells and selected metastatic variants. Evidence that this applies within a single tumor without application of selective pressures to isolate variants comes from the work of Dexter, et al., (1978). They isolated lines differing in expression of tumor-associated cell surface antigen, karotype, morphology and growth properties. The demonstration of such heterogeneity has profound implications for immunotherapy. An immune response, protective for the host, might operate efficiently only against those tumor cells with significant amounts of tumor associated antigen. Certainly the rationale for continued study of combined modalities of treatment is strengthened by such ovservations.

B. Interferons are low molecular weight glycoproteins produced by mammalian cells - classically in response to viral infection but also in response to unrelated stimuli. Now known to be produced by at least two cell types in vitro, fibroblasts and leukocytes, interferons are the focus of increased attention.

The studies of Stewart, et al., (1978) indicate that murine fibroblast interferon and human leukocyte interferon appear to have two components in vivo, one of which may be precursor to the other. However, a major problem in detailed characterization of the molecules is the small amount available for study. A key development to further study is therefore the ability to produce interferons in larger quantities. For example, Tan, et al., (1978) and Berthold, et al., (1978) report the discovery of a fibroblast cell line capable of producing 10^4 reference units of human interferon per square centimeter of culture representing more than a ten-fold increase over other lines. This has enabled the group to purify the interferon produced by these cells to homogeneity. By use of this and similar lines grown in containers with large surface areas, it should be possible to better chemically characterize these molecules. Increased production of interferons will facilitate further exploitation of the promising preliminary studies of Merigan, et al., (1978). They noted a favorable clinical response to interferon in three previously untreated patients with nodular, poorly differentiated lymphocytic lymphoma. The failure to note a similar favorable response in patients with histiocytic lymphoma adds the appropriate note of caution, but the results of other trials will be anxiously awaited.

C. Natural killer (NK) cells are providing a focus for several important areas of tumor immunology. Consensus is now apparent against the concept of a major immune surveillance role for thymus-dependent immunity during development of naturally occurring tumors of low antigenicity (Stutman, 1978). Much speculation now centers on a surveillance role mediated by NK cells as athymic mice show a correlation between in vitro NK activity and in vivo resistance to transplanted tumors. Further, the incidence of "spontaneous" tumors and chemically induced tumors in mouse strains with NK activity but no thymus dependent immunity (eg. nude mice) is no higher than in strains with both defense mechanisms intact.

Mechanistically, the natural killer cell presents some interesting puzzles. Unlike thymus dependent cell mediated cytotoxicity, there is no restriction placed on NK activity by histocompatability type - in fact NK cells can lyse targets of other species. While NK cells do show patterns of specificity when tested against panels of normal and tumor-derived cells, the breadth of their reactivity is a probable indicator of a recognition mechanism quite different from that of bone marrow and thymus dependent lymphocytes. Early indications that NK cells acted via an antibody-dependent cell-mediated cytotoxic reaction have been expanded and while such a mechanism exists under certain circumstances, NK activity in the absence of antibody has also been demonstrated. For example, Koren and co-workers (1978) have shown normal NK activity in humans with x-linked aggammaglobulinemia who lacked effector cells in antibody-dependent reactions. The development of procedures and reagents permitting the isolation of NK cells will provide an important asset to studies on the mechanism of killing.

Modulation of humoral and cell-mediated immunity by interferon has been known for some time. Recently, it has been shown that NK activity can also be effected by interferon and by agents that induce interferon. Wigzell and colleagues (1978) have demonstrated that mice injected with purified interferon

or exposed to chemical and viral inducers of interferon show a marked increase in splenic NK cell activity. Antiserum which reacted *in vivo* with interferon could block this stimulation. Trinchieri and Santoli (1978) extended this observation to humans. They demonstrated that when lymphocytes are cultured with some tumor cells, interferon is released into the culture supernatant. The interferon could then act to enhance human NK activity several fold. Interestingly, interferon could simultaneously increase the resistance of normal fibroblast targets but not of tumor cells to NK-mediated lysis. Susceptibility to lysis by thymus-dependent cell-mediated cytotoxicity did not change in the presence of interferon. Such data suggest means by which normal cells can resist the broadly specific NK activity *in vivo*.

The studies of Paige, *et al.*, (1978) suggest that cells showing spontaneous cytotoxicity may be members of a complex family. They have described a naturally cytotoxic (NC) cell which resembles NK cells closely but is not identical. It is not unreasonable to expect that a variety of sub-populations exists which are under complex regulatory control as is true of bone marrow and thymus-dependent lymphocytes.

Further study of natural killer is certain to provide exciting and important information of the tumor-host relationship.

D. Bone marrow transplantation as a treatment modality for leukemia, immunodeficiency disease, and aplastic anemia is under study by several groups. For leukemic patients the treatment protocol generally involves aggressive chemotherapy and/or radiation therapy to irradicate the tumor followed by hematopoietic reconstitution by transplantation of autologous or allogeneic marrow. Allogeneic transplantation presents several problems with immunological bases including graft rejection, graft versus host disease and infection while immunosuppressed. Because in leukemia the tumor involves the immune system directly, aplastic anemia, beside holding intrinsic interest, offers a simpler model in which to approach these problems.

We are now at the point where bone marrow transplantation is the treatment of choice for aplastic anemia in cases where a matched donor is available. For example, the Seattle team has recently summarized the results of bone marrow transplantation in one hundred and ten cases of advanced aplastic anemia (Storb, *et al.*, 1978). Fifty patients were reported as surviving between two and seventy-three months post transplant with forty-one being fully rehabilitated. Twenty-six of the study population rejected their grafts and thirty-one developed graft versus host disease. Nine of this latter group are alive with chronic graft versus host reactions. A major cause of rejection of the graft appears to be prior sensitization to minor histocompatability antigens thru supportive transfusions. One potential solution to the sensitization problem is obviously transplantation earlier in the course of the disease but a second alternative involves more aggressive preparative regimens to eliminate the sensitized lymphocytes of the recipient. Attempts are being made to better define the immunological recognition mechanism involved. Torok-Storb, *et al.*, (1978) have demonstrated that mononuclear cells from dogs which have previously been transfused can inhibit erythroid colony formation by marrow cells *in vitro* while mononuclear cells from untransfused canines can stimulate colony formation. An important technical advance that

will facilitate further analysis of this problem is the development by Dexter and colleagues (Moore and Dexter, 1978) of an in vitro culture system for long term pluripotential stem cell replication and hematopoeitic differentation. Dissection of the array of soluble factors and cells involved will be feasible only in such an in vitro system and then definitive mechanistic studies will be possible. One area that should prove interesting is the relevance of natural resistance to hematopoietic grafts studied in mice to graft rejection in man and the relationship of natural killer cell activity to both.

E. The application of in vitro cell fusion techniques to produce hybrid cells capable of secreting monoclonal antibodies provides a technical advance which has profound implications for many areas of research. Presently, the ability to produce specific antisera to a wide variety of antigens through appropriate immunizations and subsequent absorptions has resulted in quantitive and qualitative assays important to both research and clinical medicine. Monoclonal antibodies, because they consist of a homogeneous population of identical molecules with identical specificity and affinity, open the way for even wider application. While the basic method of hybridoma production is quite simple, many technical questions relating to pre-selection of parental cells, compatible cell matches to yield functional hybrids, cloning and screening for reactivity remain (Melchers, et al., 1978). The breadth of application of hybridoma and monoclonal antibody production to immunology alone indicates the impact of such technology, even at this early stage. For example, the generation of antibody diversity can be studied by analysis of the immunoglobulins produced by hybridoma clones representing the entire repertoire of response of an individual animal (Cancro, et al., 1978). The biological functions of immunoglobulin isotypes and sub-classes can be studied using several monoclonal proteins with similar antigen-binding characteristics (McKearn, et al., 1978). Fine dissection of the array of cell surface antigens displayed by tumor cells becomes possible using panels of monoclonal antibodies and this holds promise for cancer detection, diagnosis and therapy (Levy, et al., 1978). Hybrids produced by cell fusion using thymus-dependent lymphocytes can be used to better define the antigen recognition mechanism of this important class of cells as large amounts of material from a single hybrid clone become available (Ruddle, 1978). The rapid response of the research community in utilizing these developments will continue to receive strong support in the program.

Immunology is integral to many facets of cancer research. It is an area of intrinsic interest because of the many ways in which the immune system affects the growth and development of tumors. Also, the immune system provides an important model for wider based studies on development, differentiation and cell-cell interactions. Application of immunological techniques in other research areas such as virology and tumor biology provides the tools for studies not possible using alternative technologies. By encouraging all of these areas, the Immunology Program seeks to establish a synergism which facilitates the goals of the National Cancer Program.

Other Program Activities:

In order to encourage the rapid dissemination of research findings, the Immunology Program also supports meetings and conferences. Among those supported in whole or in part during this fiscal year are:

"Conference on B Lymphocytes in the Immune Response" - Scotsdale, Arizona October 15-17, 1978.

"Thirteenth Annual Symposium on Some Mathematical Questions in Biology" Houston, Texas - January 1979.

"Workshop on the Regulatory Role of Macrophage Products in Immunity" Brook Lodge, Michigan - March 12-14, 1979.

"Cell Surfaces and Malignancy" - Keystone, Colorado - March 18-23, 1979.

"Conference on T and B lymphocytes: Recognition and Function" - Keystone, Colorado - March 26-30, 1979.

"Gordon Research Conference on Tumor Immunology" - Plymouth, New Hampshire July 23-27, 1979.

"Third International Workshop on Mice" - Bozeman, Montana - September 6-9, 1979.

"Membranes, Receptors and the Immune Response" - Chicago, Illinois - September 23-26, 1979.

CANCER DIAGNOSIS PROGRAM

The Diagnosis Branch of the Division of Cancer Biology and Diagnosis has the major responsibility for the Diagnosis Program of the National Cancer Institute. Until summer 1978, this program operated almost entirely by means of the contract mechanism with the exception of several CREGs. With the administrative changes effected in NCI during 1978, grants in cancer diagnosis have been transferred to the Diagnosis Program from DCRRC. This change was a definite improvement and will provide the opportunity to choose the most desirable mechanism by which the research is best administered. The number of Advisory Committees for the Diagnosis Program has been reduced from four to two. The Diagnosis Research Advisory Group and the Cytology Automation Advisory Group provide advice but no longer have any review functions while the Immunodiagnosis Committee provides expertise for review of contracts but no longer has advisory functions; the Diagnostic Radiology Committee was merged with the Diagnosis Research Advisory Group.

PAST ACCOMPLISHMENTS

The Cancer Diagnosis Program has been in existence as such since 1973 although several contracts in cancer diagnosis began before that time. Most of the work completed at this time has been accomplished by contracts. Twenty-five diagnosis contracts have been terminated; their total cost was approximately ten million dollars. An early study in lung cancer screening led to the design used in the current studies at three institutions. Studies on pancreatic cancer diagnosis at three institutions have demonstrated the extreme difficulty of early detection and have provided leads toward further diagnostic improvements. Currently ultrasound, CAT scanning and retrograde pancreaticoduodenography can identify pancreatic cancers but not at a sufficiently early (localized) stage to improve treatment results. Three contracts have completed basic studies to test possible differences in nuclear magnetic resonance signals in normal and tumor tissue. By the methods employed it was not clear that NMR would distinguish between normal and tumor tissue, however, the studies helped to define the problems requiring solution if NMR imaging is to be useful in cancer detection. Two studies have attacked the difficult problem of optimal frequency of screening; several reports concerning those results have been published. Two studies have investigated chemical and immunological methods to more sensitively detect human blood in feces but no completely satisfactory procedure has been found. Two studies have been completed which dealt with improvement of x-ray imaging by removing scatter. One study, completed in 1976, dealt with proton beam absorption in normal and tumor tissue specimens as a preliminary to other proton beam imaging studies. The proton beam absorption was shown to be sensitive to slight differences in tissue composition and the results suggested possible usefulness of proton beam imaging for tumor detection. Several contracts now completed supported the development and cataloging of algorithms useful for x-ray and radionuclide imaging. Those algorithms are made available to other workers for further studies. A

data base was also developed for testing and studying x-ray algorithms. Development of an important new design for a body scanner was supported by the contract research program.

A substantial number of articles have been published in appropriate scientific journals describing work supported by the Diagnosis Program.

DESCRIPTION OF THE CANCER DIAGNOSIS PROGRAM

Formerly, when the Program was composed almost entirely of contracts, it was generally described as being comprised of five specific areas of involvement: General Cancer Diagnosis, Diagnostic Radiology, Cytology Automation, Breast Cancer Diagnosis and Immunodiagnosis. The first three areas were the province of the Diagnosis Branch, DCBD, while Breast Cancer Diagnosis was directed by the Breast Cancer Task Force of DCBD, and the Immunodiagnosis Contract Program was operated by the Immunology Program, DCBD.

After the reorganization at NCI, during 1978, when the diagnosis grants were added to the Diagnosis Program it has become convenient to classify both contracts and grants in a more unified system. The present system has eight disciplinary categories: Biochemistry, Immunodiagnosis, Cytology, Pathology, Radiological Imaging, Non-Radiological Imaging, Nuclear Medicine and Multiple Disciplines. The distribution of contracts and grants currently in the Cancer Diagnosis Program into the various categories is summarized in the following table. Each category is discussed in the sections following the table.

TABLE 1

Cancer Diagnosis Research Program

Number	Category	Contracts		Grants	
		Number	Current Funding	Number	Current Funding
1	Biochemistry	3	80,613	14	954,380
2	Immunodiagnosis	37	2,837,012	10	610,301
3	Cytology	14	2,339,192	8	1,043,108
4	Pathology	0	0	3	193,642
5	Radiological Imaging	4	1,398,953	11	885,347
6	Non-Radiological Imaging	3	690,498	9	1,050,549
7	Nuclear Medicine	3	186,741	13	961,840
8	Multiple Disciplines	16	4,329,872	2	111,524
	Totals	80	11,862,881	70	5,810,691

1. BIOCHEMISTRY

Biochemical methods to improve diagnosis and detection of cancer involve the study of a variety of substances such as hormones, enzymes, proteins and metabolic products in the circulation and in other biological fluids, as well as study of the surface characteristics of tumor cells and the chemical characterization of tumor cells. An extensive study of circulating hormones such as ACTH, vasopressin, HCG (both alpha and beta sub-units), beta-lipotropin, calcitonin and thyroid stimulating hormone under contract CB-43903 has demonstrated the level of their association with selected types of cancer. A variety of enzymes are being studied for their correlation with different cancers. Histaminase and an isozyme of alkaline phosphatase are being investigated as general cancer markers by grant CA-26652 and contract CB-74173 respectively. An improved methodology for serum acid phosphatase is being studied for diagnosis of prostatic cancer by grant CA-19231 and 5'-nucleotide phosphodiesterase has demonstrated ability to detect liver metastases in patients with breast cancer under contract CB-64029. A profile of enzyme patterns is being studied to help characterize and classify human lung cancer under grant CA-25016 and enzymes in plasma and cells are being monitored during the treatment of patients with lymphoma to provide guidance for the therapy under grant CA-22065. The protein analysis of pancreatic secretions is being studied as an aid to the diagnosis of pancreatic cancer by grant CA-14380. The chemistry of serum lipoproteins is being studied to determine whether qualitative as well as quantitative differences occur between mammary carcinoma patients, normal individuals with a family history of cancer and those without family history of cancer in grant CA-19634. A "myasthenic substance" found in some small cell lung cancer is being studied as a possible aid in the detection of lung cancer by grant CA-22885. The serum levels of a heteroglycan fraction which have been found to be markedly elevated in tumor-bearing animal models, are being evaluated for diagnostic sensitivity and reliability in cancer by grant CA-21958. Studies are in progress on the biochemical detection and identification of the entire range of DOPA metabolites and other reducing compounds in the urine of malignant melanoma patients. Patterns of these metabolites were found to differ greatly from normal DOPA metabolism in contract CB-20423. The usefulness of cyclic GMP and cyclic AMP assays is being evaluated as a potential measure of cellular proliferation and also, detection, diagnosis and treatment of estrogen-dependent neoplasms in human females by grant CA-25357. In studies on human chondrosarcomas, the structural changes which occur in the components of the intracellular matrix are being investigated as indicators of the degree of malignancy by grant CA-23945. Estrogen receptors are being studied in breast cancer and also the patient's response to endocrine manipulation by grant CA-23848, in an attempt to develop a direct method of determining fluorescent hormone uptake in hormonally dependent cancers as well as in normal target tissues such as endometrium and mammary gland. The differentiation of lymphocytes by the enzyme marker, terminal deoxynucleotidyl transferase (TdT) is also being investigated by grant CA-22599 by determination of the relationship of the presence or absence of the enzyme to particular cell sets and to tumors representing those cell sets.

2. IMMUNODIAGNOSIS

The portion of the Diagnosis Program classified as Immunodiagnosis can be subdivided into projects dealing with circulating tumor antigens or markers, projects dealing with antibodies to tumors (including immune complexes) and studies of cell mediated immunity. The carcinoembryonic antigen is the most widely utilized circulating tumor marker and there are two contracts dealing with the prognostic and monitoring value of CEA following therapy of colorectal and lung cancer: CB-23854 and CB-33854. Additionally there are studies on the heterogeneity of CEA, grant CA-24376, and comparison of new colon cancer antigens with CEA, contract CB-84259. Several projects are investigating the relationship between hormone levels and cancer; calcitonin, HCG and beta-lipotropin are subjects for three grants, CA-22137, CA-18362 and CA-23382. Two studies of serum calcitonin as a screen for family members of patients with medullary thyroid cancer are supported by grant CA-22595 and contract CB-63994. Two studies of enzymes as markers for cancer deal with tyrosinase in patients with melanoma, grant CA-25381, and UDP-galactosyltransferase in patients with ovarian and breast cancer, contract CB-84260. Proteins or their degradation products have been detected in body fluids and have demonstrated correlation with the presence of cancer. Studies of some of these are in the developmental phase, grants CA-24645 and CA-13447 and contract CB-84308, while the fetal sulfoglycoprotein antigen in gastric juice is being studied in a large detection trial for stomach cancer by contract CB-64070. New tumor associated antigen studies are under way for many organ sites: prostate, CA-23990 and CB-74169; breast CA-20286 and CB-74162; cervix, CB-84267; ovary, CB-64013 and CB-64028; leukemia, CB-74165; melanoma, CB-74127; and hepatoma in rodents, CB-74167. In addition there are contract studies to investigate the relationship between microbial products and tumor antigens, CB-74122, and the production of a lymphotoxin by lymphoblastic cells in tissue culture, contract CB-84263. Circulating antibodies to tumor antigens are studied in patients with melanoma, contract CB-74120, while the presence of circulating immune complexes are being quantitated and analyzed in a number of cancers including colon, breast and melanoma by contract CB-84262. Antibodies are also serially measured in mice while developing induced tumors, contract CB-74134. Xeno-antibodies to various tumor markers are being used to classify malignant tissues by contract CB-84257 using immunohistochemical techniques. Various parameters of cell mediated immunity are being studied under contracts to define the functional activity of monocytes, CB-74121; the cryopreservation of lymphocytes and monocytes with cytotoxic activity, CB-74131 and CB-74164. The definition of subpopulations of leukocytes using separation techniques depending on specific antibodies or lectins is the subject of three studies: CB-74132, CB-74163 and CB-74129.

Studies of natural cytotoxic activity of leukocytes are supported by two contracts, CB-74133 and CB-74166, and the augmentation of cytotoxic activity by another contract, CB-74130. An evaluation of the leukocyte adherence inhibition assay for cancer diagnosis is being performed by contract CB-80316. In addition to the research grants and contracts there are contracts for the collection and storage of serum samples from patients with cancer and other acute and chronic diseases and during the course of therapy for certain

cancers. These contracts, CB-33914, CB-74124, CB-74210 and CB-84258, have been invaluable for the rapid evaluation of a wide variety of serologic tests for cancer that come to the attention of the NCI. A tissue culture bank for cell lines which can be utilized by an investigator for research in cancer immunodiagnosis is maintained under a contract, CB-43854.

3. CYTOLOGY

Diagnostic cytology research projects include the development of automated instrumentation and cell markers that can be used to differentiate normal and atypical cells. Instruments undergoing development and testing are those of high resolution slide based, grant CA-13271 and contract CB-33873, and flow fluorometric types. Automated screening for abnormal cells involves examination of large numbers of cells. Development, construction and testing of an ultrafast optical scanner for microscopic specimens, grant CA-24466, will make possible the automated search for abnormal, transformed or cytochemically marked cells in microscopic preparations. Single cell classification algorithms employing the current state of the art in digital image processing, scene segmentation and image acquisition hardware are being developed and tested, contract YO1-CB-70314. The classification algorithm chosen with human interaction was found to perform at an acceptable error rate. Flow instruments currently undergoing testing use dual staining and analysis with dual laser beam sorters, contracts YO1-CB-60311 and YO1-CB-40300. In addition, a slit-scan using a combined static and flow system is now being tested to determine the system characteristics including rate and causes of false alarms, contract NO1-CB-33862. Quantitative descriptors of normal and abnormal cells include cytochemical and immunological markers which increase the potential sensitivity and specificity of sensor systems. Chemical synthesis of cytochemical probes, grant CA-24363, with sharp fluorescence emission spectra will enhance multiple staining of cells for flow systems and for cells on slide in static systems. Cellular fluorescent markers under investigation also include studies of fluorescent substrates for demonstration of cAMP phosphodiesterase, adenyl cyclase, and acid phosphatase, grant CA-19552; acridine orange, contract CB-33862; and other DNA protein fluorescent dyes, contracts YO1-CB-40300 and YO1-CB-60311. Cell surface antigens may also be used as markers for abnormal cells. With appropriate fluorescent tagging of these antigens, normal and abnormal cells may be differentiated by automated instruments. Potentially useful markers being studied under contracts are total HSV viral antigens, AG-e, contract CB-74170, antigens extracted from HSV-1 and HSV-2 transformed cells, contract CB-74171, and a cervical carcinoma antigen, CCA, contract CB-74096. In order to analyze the performance of automated systems work the comparison of instrumental or system classification and manual microscopic classification utilizing standard morphologic criteria has been started under contract CB-74190. In addition, programming of cell recognition algorithms continues with emphasis on scene segmentation techniques in the same contract. Research is in progress, contract CB-74095, to test a module sorter to separate cells based on individual cell parametric measurements. This module will be interfaced with a flow instrument, contract CB-33862, to assure a correlation of individual separated cells with specific quantitative measurements. To better understand and treat lymphoproliferative disease,

flow microfluorometry analysis of human lymphoid malignancies is under investigation, grant CA-23393, with the hope that malignant subpopulations can be identified by two parameter analysis for size and monoclonal surface immunoglobulin. In addition, it is hoped that the ploidy and cell cycle kinetic parameter of these cells will aid in the diagnosis, classification, scheduling and monitoring of treatment. Leukemic cells are also being studied to determine their proliferation and differentiation in semi-solid matrix culture using cytologic markers, to correlate markers of abnormal growth with clinical alterations, and to test chemotherapeutic agents for their ability to alter patterns of cell differentiation in vivo, grant CA-22942. Study of human leukemic and pre-leukemic blood and bone marrow using the soft agar culture technique has continued, grant CA-17353, to characterize the status and prognosis of hematoproliferative disorders. Measurements of the quality and quantity of colony formation appear to provide useful indicators of disease status and progression as well as diagnosis and predictions concerning response to therapy of acute non-lymphocytic leukemias and preleukemic states. In an effort to predict the biological potential of gynecologic neoplastic lesions, a quantitative analysis of nuclear DNA content using Feulgen microspectrophotometry is under way, grant CA-24932. Determination of the nuclear DNA changes may reveal the correlation and association of the changes with regression, persistence and progression of squamous cancer of the uterine cervix and its precursors.

4. PATHOLOGY

Identification and enumeration of all lesions from whole breast using a subgross method showed elevated frequencies of atypical lobules and other microscopic lesions in breasts of patients with cancer. Conclusion was made that almost all lesions are located in the terminal ductular alveolar region and that certain specific lesions have prognostic importance, grant CA-21523.

A system of classifying human pituitary adenomas has been developed, by grant CA-21905, based on electron microscopy and immunocytochemistry, which recognizes as distinct entities: 1) growth hormone cell adenomas, 2) prolactin cell adenomas, 3) mixed adenomas consisting of growth hormone cells and prolactin cells, 4) corticotroph adenomas, 5) thyrotroph adenomas and 6) undifferentiated cell adenomas. To expand our understanding of tumor behavior in the pyriform sinus and the oral cavity in order to better preserve important regional functions after cancer surgery, whole organ sections of patients who undergo surgical excision have been studied, by grant CA-22101, using microscopic appearance to determine the size and extent of tumor, relation of tumor spread to the laryngeal framework and specific routes of tumor spread.

5. RADIOLOGICAL IMAGING

Several contracts and grants to improve x-ray imaging currently are under study. Two contracts, CB-74112 and CB-74211, seek to develop large area solid state image receptors for x-ray imaging. A grant, CA-16543, is studying charge transfer electroradiography. Contrast agents to improve CAT scanning studies

of the liver, contract CB-84234, development of algorithms for x-ray dose reduction in CT scanning, contract CB-84235, image reconstruction from incomplete projections, grant CA-23818, study of optical quality effects on lung tumor detection, grant CA-23816, and study of diagnostic accuracy and x-ray image properties, grant CA-24625, are all examples of efforts to improve imaging and reduce x-ray exposure or both. Improved imaging of breast tumors is the subject of several grants: CA-22803, computer evaluation of mammographic calcifications; CA-25372, new fluorescent x-ray tubes for mammography; and CA-19622, low dose screening techniques for mammography. Other grants related to radiological imaging include CA-15882, construction of a transverse section x-ray camera; CA-21452, screening for neoplasms in irradiated populations; CA-23246, recognition of very small tumors; and CA-24822, study of a 3-D tomogram viewer for cancer detection and therapy.

6. NON-RADIOLOGICAL IMAGING

Imaging studies in the program involving methods other than x-ray include the use of proton and heavy ion beams, ultrasound, thermography, and electron paramagnetic resonance (EPR). Contract YO1-CB-40302 supports a study of the imaging potential of proton, helium, and carbon ion beams; extremely small tissue density differences are detected by the ion beams but lateral resolution is inferior to x-ray. A study of EPR imaging in cancer detection is being carried out on grant CA-14335. Imaging with thermography at microwave and millimeter wavelengths is being studied on grant CA-17642 while grant CA-23142 is concerned with thermography enhanced by microwave and acoustic energy irradiation. Several grants are investigating means to improve breast imaging with ultrasound: CA-19019, ultrasound mammography; CA-23616, new ultrasonic methods for breast cancer detection; CA-24085, quantitative ultrasound imaging of the breast; CA-24257, breast tumor diagnosis by computed ultrasound; CA-25323, ultrasonic computed tomography of breast cancer; and CA-25634, development of an ultrasonic breast tissue phantom. Contrast agents for ultrasound diagnosis are being developed in contract CB-84236 and an ultrasonic probe suitable for use in endoscopes is the goal of contract CB-74136.

7. NUCLEAR MEDICINE

The major thrust of grants and contracts in the Cancer Diagnosis Program which use radionuclides concern tumor-seeking or tumor-delineating agents. Several grants are devoted to production of a variety of tagged tumor-seeking agents: CA-08349, CA-15787, CA-16861, CA-18153, CA-19898, CA-24344, and CA-25739. Contracts CB-64011 and CB-64073 and grant CA-17742 deal with production of, and localization studies with labelled immunoglobulins against CEA. Grant CA-22077 is studying zinc radiopharmaceuticals for ocular melanoma detection; grants CA-22578 and CA-24597 deal with imaging melanomas in general. Grant CA-24879 is studying malignant perfusion applied to diagnosis and therapy. Contract YO1-CB-50304 has been concerned for several years with production and collection of algorithms for use in radionuclide systems and the development of a user's manual to facilitate their utilization by other investigators. Grant CA-17731 is devoted to development of coded aperture systems for nuclear dose

reduction.

8. MULTIPLE DISCIPLINES

A number of contracts and a few grants in the Cancer Diagnosis Program involve several disciplines and therefore are presented in this last category of multiple disciplines. Five contracts, CB-43910, CB-43980, CB-43981, CB-43982, and CB-43983, have spent five years in a collaborative study to evaluate computerized tomographic x-ray imaging for cranial tumors and compare this with radionuclide imaging. Those studies are now completed and reports are being written for publication. Contract CB-84232 has begun studying various diagnostic techniques including imaging methods to improve pancreatic cancer diagnosis. Grant CA-14712 is involved with investigation of high risk factors in breast cancer. Contract CB-64010 has developed a standard protocol for evaluation and comparison of imaging methods. Radionuclide markers for tumors on or near endoscopically approachable surfaces and appropriate detectors for them which can be used in an endoscope are being developed on contract CB-64012. Grant CA-25582 is studying fluorescence endoscopy and photoradiation therapy with porphyrin derivatives. Contracts CB-74114 and CB-74212 are developing attachments for the colonoscope to facilitate its easier passage to the cecum. A collaborative study to determine the potential of x-ray imaging and cytological examination of sputum in detection of early lung cancer is being conducted under contracts CB-45007, CB-45037, and CB-53886. A fourth related contract, CB-43868, is concerned with the data management of the lung study. The longitudinal study began in 1974, involves 30,000 men, and will require several more years of follow-up before definitive results will be available. The feasibility of using existing methods of endometrial sampling as a means of screening women for endometrial cancer is being studied on contract CB-84233. A large scale longitudinal colon cancer screening study using the Hemoccult test for human blood in the feces is the task of contract CB-53862. Several more years of follow-up on the 45,000 subjects will be required to define the usefulness of the screening procedure in detecting early colon cancer.

A major activity during the year has been to establish grants as an integral part of initiating and promoting the extramural breast cancer research program. The number of grant applications related to epidemiology, experimental biology, diagnosis and treatment aspects of the disease have increased at each cycle of the Division of Research Grants Receipt and Referral process. Also, the number of grants funded have increased, however, there have been fewer with fundable scores than had been anticipated a year ago. For FY 1979 approximately $2.0 million have been utilized for new project grants (R01), conference grants (R13) and young investigator awards (R23). The majority of the grants funded represent the area of experimental or basic biology.

The Breast Cancer Task Force (BCTF) Committee whose membership is separated into extramural expertise for four Working Groups, Experimental Biology, Epidemiology, Diagnosis and Treatment have devoted considerable effort to becoming familiar with the on-going grants, to planning projects that require new or increased emphasis, and to determining the preferred means for stimulating new programs, e.g., workshops, conferences, RFAs, and for program announcements. A Program Announcement has been formulated that is aimed at increasing present knowledge of radiation-induced risk for breast cancer in humans.

The number of contracts in the program have been reduced from 96 at the beginning of FY 1979 to 80 in the ensuing nine months. The total funding has been decreased by $2.0 million. During FY 1980 approximately one half of the on-going contracts will be phased out. However, the contracting mechanism will continue to be utilized for obtaining research resource materials and for projects where planning and monitoring by staff are required. On-going service and support contracts will be continued for: data collection and analysis of marker studies (CB-74140); human cell and animal tumor bank (CB-74175); preparation of antibodies to collagen and procollagen (CB-84225); storage of human sera (CB-74213, 74138, 84296, 74137).

Contracts representing basic research in the biology of the mammary gland, normal and abnormal, have been supported strongly in the past by the breast cancer program. These projects are closely related to a number of on-going investigator-initiated grants and in fact the grants represent many of the same research objectives of our program in experimental biology, therefore many of these contracts have been or will be phased out in the coming year. During FY 1979 several of the principal investigators for terminated contracts have applied for grants and have been funded. The Experimental Biology Working Group and two other Working Groups of the BCTF Committee continue to emphasize the need for successful culturing techniques for normal mammary epithelial cells. The staff of the Branch and advisory members are planning a Workshop on this topic for the coming year. Following a review of the experimental biology program the members of the Working Group have advised that the animal mammary tumor and human breast cancer cell bank facility be made more visible to the scientific community through publicity

in scientific journals. Another recommendation has been to contract for the preparation of resource materials such as: human, rat, and mouse alpha-lactalbumin, casein and antisera for each; and, antibodies to mammary epithelial cells. For investigator-initiated grants the following new topics have been suggested for RFAs: 1) Studies of the mechanism by which protein and low caloric diets dramatically reduce the incidence of spontaneous mammary tumors in mice and in chemically-induced mammary tumors in rats; 2) Study of the epidemiology of breast cancer among free-living rats and mice captured in various environmental settings; 3) Determine the relationship of anterior pituitary hormones to breast cancer; 4) Identification of cytosol and nuclear bound estrogen in breast cancer cells using fluorescent spectroscopy or electron probe analyses and correlates with receptor levels and steroid responsiveness.

In the area of epidemiology our program has supported research on families with high incidence of breast cancer and primarily because of the importance of a possible genetic component in breast cancer susceptibility. Included in the program have been biochemical analyses of a large number of markers and further analysis for possible linkage between any of the known markers and a "susceptible allele" for breast cancer. This research (CB-44003, 84274) has recently uncovered a probable linkage of breast cancer susceptibility to one of the markers, namely, glutamine pyruvate transaminase. Because of the exciting possibilities opened up by this breakthrough, the Epidemiology Working Group sponsored a Workshop (May 1979) to present and discuss an up-to-date evaluation of genetic aspects of breast cancer. As a result of the meeting a program announcement soliciting further research in this area is being drafted. Another area in which data are proving to be of considerable interest is that on estrogen receptors (ER) in conjunction with pre- or post-menopausal status, pathological classification of the tumor, and a variety of epidemiological characteristics of the patient (CB-53884, 63996, 63998, 84319). These data will allow the examination of the epidemiology of estrogen receptors. Three projects are being formulated for RFAs: 1) A pathology study ignoring conventional classification and aimed at looking for possible differences between breast cancer tissue from patients who died within three years of diagnosis and tissue from patients who were still living 10 years later. This would attempt to explore further, known heterogeneity in breast cancer malignancy and patient survival. 2) A search for additional, presently known factors by a case-control study of women who are at low risk for the disease, according to known risk factors (no family history of breast cancer, no benign breast disease, early first pregnancy, etc.), but who never-the-less develop the disease; 3) In depth study of the relationship between infertility problems, luteal phase hormonal abnormalities, and risk of breast cancer.

Two contracts initiated in FY 1978 in the area of diagnosis have produced potentially important data. One study (CB-84273) involves clinical applications of ultrasound scanning for breast cancer detection. Two "State of the Art" ultrasound machines, the Ausonics Octoson and the Johnson and Johnson real-time scanner are being evaluated clinically as a possible alternative or adjunct for x-ray mammography. The controversy over radiation risk qualifies this project a high priority endeavor. The other study (CB-84228) probes the immunological response of cells from the regional lymph nodes as

a prognostic indicator. Marked differences have been found in some breast cancer patients' effector cell populations as well as in proliferative responses and lymphokine production of lymph node cells compared to those of control subjects. The findings indicate tumor related immune stimulation. In a long-term contract (CB-43869) final data are being analyzed on establishing the use of computerized, automated Absolute Temperature Thermography with pattern recognition techniques as a valuable pre-screening device. An 89 percent sensitivity rate with an associated 75 specificity rate has been developed with computer techniques on a population sample of 407 normals and 72 cancer subjects. It is anticipated that the results will not diminish and will justify consideration of the use of this non-invasive technique for clinical trials in a mass screening setting. The Diagnosis Working Group has recommended several new projects for future study: 1) Effect of diagnostic ultrasound exposure on the induction or enhancement of breast cancer tumor growth and metastases. 2) Isolation and characterization of infiltrating lymphoreticular cells from human breast tumor tissue. 3) Immunological and biochemical characterization of agressive vs non-aggressive breast carcinoma types. Also, the Working Groups has suggested several important topics for future workshops.

On going contracts in the treatment of breast cancer have been desirably productive in this fiscal year. In a surgical adjuvant study involving 296 women with histologically proven Stage II cancer of the breast, preliminary results show a significantly lower tumor recurrence rate in the patients with ER positive cancers who receive cytotoxic chemotherapy, Cytoxan, Methotrexate and 5-Fluorouracil (CMF) plus the anti-estrogen tamoxifen (T) than in those who receive only CMF. The patients with ER negative tumors show no difference in recurrence rates between the CMFT and CMF treatment groups (CB-43990). In another project the investigators have established an effective well tolerated regimen of adrenal suppressing aminoglutethimide (AG) which appears to be an appropriate alternative to surgical adrenalectomy for the treatment of advanced breast cancer (CB-53851). The Data Center (CB-74140) for the Breast Cancer Treatment program has abstracted and stored clinical and estrogen receptor assay data on 2200 patients and clinical data on more than 6000 patients whose serum is being stored for future biological markers studies. Of major importance has been the preparation of the first definitive antibodies to estrogen receptor after obtaining purified nuclear estradiol-receptor complex. Progress is underway with potentially more efficient procedures, affinity chromotography and hybridoma cell lines (CB-43969). In a retrospective study investigators are restudying axillary lymph nodes from 500 patients with previously reported histologic Stage I breast cancer and correlating their findings with the 5 to 10 year clinical outcome of these patients. The results are expected to determine whether detailed microscopic study contributes significantly to the prognostic accuracy (CB-77110). Suggestions for workshops or overviews that may lead to RFAs or program announcements have been made by the Treatment Working Group. For example: 1) Immunological aspects of breast cancer that would include the immunocompetence profile of the patient with breast cancer prior to and after surgery, during chemotherapy and general course of the disease. 2) Cosmesis of surgery to review the efficacy of procedures other than radical mastectomy and compare the acceptability of cosmesis obtained by wedge resection, radiotherapy, reconstructive surgery and combinations of limited surgery and

irradiation. 3) Chronobiology as it is related to treatment following mastectomy and recurrence of the disease. 4) Minimal breast cancer, consideration of definition, radiographic, histopathological and clinical features.

The Information Section of the Branch has become productive and extremely useful to staff, BCTF Committee members, and investigators who have made specific requests for various types of information. Intracom which is a more useful version of Intercom is being produced monthly. It provides global coverage in abstract form of the published literature on breast cancer and grant and contract activities. Various reports were generated from the Medlars system in response to specific inquiries. Teletype capability has been established, the terminal is flexible and can be configured easily to communicate with other ASC II terminals at speeds up to 120 characters per second. It functions either in the keyboard mode for interactive communication or for automatic send/receive of brief documents. A minicomputer installation was completed in March 1979, programming necessary for communication with the IBM 320 System at the National Library of Medicine has been completed and in test runs all components perform flawlessly. The computer is interfaced to the Bell Switched Network, i.e., it can communicate with a terminal anywhere in the world via ordinary telephone line or data transmission networks. Intracom production on microfiche has been a primary reason for acquiring the minicomputer. Microfiche production will make it possible to send the abstracts of publications to investigators working in breast cancer throughout the world at a reduced cost in paper and mailing costs.

Meetings:

A group of basic and clinical scientists were brought together at the request of the Director, NCI, to discuss retinoids and breast cancer on November 14, 1978. The consensus was that additional animal experimentation was required prior to initiating human clinical studies.

A Consensus Meeting "Steroid Receptors in Breast Cancer" was held in Bethesda, June 27-29, 1979. The purpose of the meeting was to review and discuss current information on clinical uses of steroid receptor assays of breast cancer tissue. Based on presentations made by over 50 investigators from the U.S.A. and several continents, a report was formulated and published in which those applications of ER found to be valid and useful for the management of breast cancer patients were discussed.

The BCTF Committee met in February and May, 1979 in open sessions, the new extramural Chairman, Dr. Eugene R. DeSombre, University of Chicago, presided.

Publications

Levine, B. S., DeSombre, E. R., Taylor, D. J. and Rabson, A. S.
Breast Cancer Task Force Program and Related Projects.

IMMUNOLOGY CONTRACT PROGRAM

CONTRACT RESEARCH SUMMARY

Title: Intrapleural BCG After Primary Surgery for Lung Cancer

Principal Investigator: Dr. Martin F. McKneally
Performing Organization: Albany Medical College
City and State: Albany, NY

Contract Number: NO1-CB-53940
Starting Date: 6/1/75 Expiration Date: 9/30/80

Goal: To conduct preclinical and clinical evaluation of the therapeutic efficacy of intrapleural BCG with or without tumor antigen preparations in Stage I, II and III lung cancer.

Approach: Following surgical resection of their tumors, patients with lung cancer had been randomly assigned to receive intrapleural BCG plus INH or INH alone. Subsequently, the design was altered so that test Stage I patients now receive BCG plus INH followed by additional supplemental doses of BCG, while control patients received the BCG plus INH but no supplement. Stage III test patients now receive BCG + INH + supplemental BCG; the control patients receive no immunotherapy after surgery.

Progress: Among 30 Stage I patients treated in the first trial of intrapleural BCG and oral INH, there have been 8 recurrences of lung cancer, one new primary cancer, and 2 non-cancer deaths. Among 36 simultaneously entered control (INH) patients, there have been 19 recurrences of lung cancer, 3 new primary cancers, and one non-cancer death. Statistical analysis has demonstrated a significant difference between the BCG-treated Stage I population and their INH-treated counterparts; P=0.006.

Thirty-seven patients have been entered into the second Stage I study. Of 22 patients who received a single intrapleural injection of BCG, 4 have had recurrences and 1 patient died on the 19th postoperative day of an anaerobic bacterial empyema. All 15 patients who received intrapleural and cutaneous BCG are well and free of disease. The median duration of follow-up in this phase of the study is 14 months.

Significance to Cancer Research: This randomized control trial shows a statistically significant treatment effect due to immunotherapy. A repeat trial with larger numbers of patients is being conducted to confirm the result.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $110,514

CONTRACT RESEARCH SUMMARY

Title: Procurement of Large Volumes of Sera from Cancer Patients

Principal Investigator: Dr. John J. Lynch
Performing Organization: Bio Med, Inc.
City and State: Washington, DC

Contract Number: NO1-CB-74125
Starting Date: 9/30/77 Expiration Date: 5/29/79

Goal: Collection of 200 ml of plasma from donors with gastrointestinal cancer (36), melanoma (10), lung cancer (25), and breast cancer (25) as well as control specimens for use in immunodiagnostic studies.

Approach: Collect clinical data at the time of plasmapheresis. Store plasma in aliquots at -70°C or ship to specified recipients.

Progress: New contract.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Cells Involved in Immune Response to Tumors

Principal Investigator: Dr. Robert Stout
Performing Organization: Brandeis University
City and State: Waltham, MA

Contract Number: NO1-CB-64034
Starting Date: 6/15/76 Expiration Date: 6/30/79

Goal: To develop a system for stimulation of isolated amplifier T cells so as to allow amplification of cytotoxic responses to syngeneic cells which bear tumor-specific antigens but no allogeneic Ia antigens.

Approach: The approach entails a systematic study of the effect of mixed lymphocyte and Con A-stimulated subpopulations on the cytotoxic responses of unstimulated subpopulations of T cells to allogeneic and syngeneic tumor cells. The subpopulations of T cells will be detected by the presence of surface Ly or FcR determinants and isolated on a Fluorescence Activated Cell Sorter.

Progress: All required culture and assay systems have been established and key reagents have been prepared and tested. Conditions for optimal stimulation of MLR and CML responses in microculture have been determined. MLR proliferative responses of 2×10^5 responder lymphocytes increased as the number of stimulator cells was increased to 16×10^5 cells. Lymphocytic responses in similar cultures increased as the number of stimulators was increased to 4×10^5 cells and decreased thereafter. The ability to isolate the interacting T cell populations for CML responses in the B6 mouse system on the basis of the presence or absence of the FcR (Fc receptor) was confirmed. Optimal CML was generated only when both FcR-negative and FcR-positive responder T cells were present in culture. MLR-activated T cells can, when added to fresh cultures, increase the subsequent CML response. This increase ranged from 1.5- to 4-fold in different experiments when 1×10^5 MLR-activated cells were added to 4×10^5 fresh responder cells. Irradiated MLR-activated T cells can also function in this manner, but culture supernates from MLR cultures do not seem to contain an enhancing factor.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Production and Detection of Antibodies to Chemical Carcinogens

Principal Investigator: Dr. Helen Van Vunakis
Performing Organization: Brandeis University
City and State: Waltham, MA

Contract Number: NO1-CP-23243
Starting Date: 4/20/72 Expiration Date: 1/31/80

Goal: To develop immunoassays that can be used to determine carcinogens, procarcinogens, cocarcinogens, and their metabolites in physiological fluids, tissue extracts, and other material. Such assays also facilitate studies on pharmacokinetics and metabolic conversions, and are useful in the characterization of enzyme systems.

Approach: The compound is coupled to a macromolecular carrier that promotes its antigenicity and the resulting conjugate is used for immunization of animals. Resulting antibodies are used to devise radioimmunoassays.

Progress: During the current contract period an assay for N-nitrosonornicotine (NNN) has been developed and used to follow the disposition of NNN in the physiological fluids of rabbits and examine the sera of smokers. The contractor has obtained antibodies specific for nornicotine. Analysis of human saliva and sera shows that saliva can be used to determine smoking status. In vitro experiments have shown that nicotine and cotinine analogues of nicotinamide dinucleotide can inhibit certain dehydrogenases. Most recently the contractor has analyzed tissue samples of two smokers and three non-smokers for nicotine and cotinine analogues of nicotinamide mononucleotide and nicotinamide dinucleotide. The analogues were only detected in the smokers' tissue samples.

Significance to Cancer Research: In addition to monitoring levels of noxious agents in the environment, the availability of such assays facilitates basic studies on carcinogens, procarcinogens, and cocarcinogens that may help us understand how they exert their biological activities.

Project Officers: Dr. Herbert Rapp and Ms. Judith Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $138,000

CONTRACT RESEARCH SUMMARY

Title: Induction of Colonic Tumors in Guinea Pigs

Principal Investigator: Dr. Gary L. Cockerell
Performing Organization: Cornell University
City and State: Ithaca, NY

Contract Number: NO1-CP-65744
Starting Date: 9/30/76 Expiration Date: 9/29/80

Goal: To produce carcinomas of the colon in approximately 700 NIH guinea pigs and subsequently test the immunotherapeutic effect of BCG vaccines versus other modalities of treatment.

Approach: N-methyl-nitrosourea is administered by intrarectal installation to produce colonic carcinomas. Following tumor induction, laparotomies are performed, and the tumorous portion of bowel is (1) injected intralesionally with BCG vaccines, (2) surgically resected and a diverting colostomy established, or (3) located but not otherwise treated. Animals are then allowed to recover from surgery and are observed until death.

Progress: More than half of the animals have been entered into the study. Preliminary results indicate that surgical resection of the colon cancer may prolong survival of the animals. So far intralesional injection of emulsified BCG cell walls has not prolonged survival. It is still too early, however, to draw any firm conclusions about the efficacy of surgery versus immunotherapy in the treatment of chemically induced colon cancer in guinea pigs. Studies are in progress on the development of methods for early diagnosis of the experimental colon neoplasm and for studying the immunologic aspects of tumorigenesis.

Significance to Cancer Research: The guinea pig colon carcinoma model will serve as a useful system to test various methods of tumor treatment, since it has now been shown that the guinea pig colon tolerates intramural BCG injection and is surgically resectable.

Project Officers: Dr. Herbert Rapp and Ms. Judith Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $249,741

CONTRACT RESEARCH SUMMARY

Title: Long-term Tumor-specific Cytotoxic Lymphocytes in Treatment of Mouse Leukemia

Principal Investigator: Dr. Kendall A. Smith
Performing Organization: Dartmouth Medical School
City and State: Hanover, NH

Contract Number: NO1-CB-74141
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: To evaluate the usefulness of tumor-specific cytotoxic lymphocytes maintained in continuous culture for the prevention and treatment of mouse leukemia.

Approach: Attempts will be made to (1) demonstrate the tumor-antigen reactivity of human and murine cytotoxic T-cell lines; (2) test in vivo the immunoprophylactic and immunotherapeutic value of murine cytotoxic T cells maintained in continuous proliferative culture; and (3) characterize and purify the growth factor which allows for the long-term culture of cytotoxic lymphocytes.

Progress: Of 24 clones derived from a murine cell line (CTLL-2) which mediated both alloantigen- and tumor antigen-specific lysis, 10 clones mediated only alloantigen-specific lysis, 4 clones mediated only tumor antigen-specific lysis, 6 clones lysed both alloantigen- and tumor antigen-bearing targets, and 4 clones displayed no cytotoxic activity. In Winn assay trials the cloned tumor antigen-specific CTLL retarded the growth rate and prolonged survival of mice inoculated with a lethal dose of poorly antigenic, syngeneic tumor cells.

Three alloantigen-specific human cytotoxic T-lymphocyte lines (H-CTLL) have been maintained in culture for more than 8 months. In an initial human mixed tumor-lymphocyte culture effector cells were generated capable of lysing autologous tumor cells.

Studies have continued on the biochemical and immunobiological function of the T-cell growth factor.

Significance to Cancer Research: The ability to cultivate large quantities of tumor-specific cytotoxic lymphocytes which retard in vivo tumor growth may provide a new approach to cancer treatment. The successful culture of differentiated functional T cells for extended periods offers the promise of new understanding of the mechanisms of cellular immunity and of immunodeficient and hyperimmune disease states.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $104,100

CONTRACT RESEARCH SUMMARY

Title: Molecular Studies of T Cell-mediated Cytotoxicity

Principal Investigator: Dr. Jeffrey R. Dawson
Performing Organization: Duke University
City and State: Durham, NC

Contract Number: NO1-CB-74152
Starting Date: 9/30/77 Expiration Date: 9/29/79

Goal: Study mechanisms of T cell-mediated cytotoxicity at the molecular level.

Approach: Use as a model system the specific killing of allogeneic tumor cells by immune peritoneal exudate cells (CHY and EL-4 tumor cell lines). Spin labeling will be applied to studying cell interactions.

Progress: This study focuses on T cell-mediated cytotoxicity of two tumors, EL-4 and CHY, as a model system in which to investigate the mechanism of killing. The lines are both leukemias of C57BL mice but differ in several respects. CHY is surface Ig positive and Thy 1.2 negative indicating a B cell origin, while EL-4 appears to be a TR cell leukemia. CHY is less susceptible than EL-4 to lysis by antibody plus complement, by cytotoxic T lymphocytes and by antibody-dependent cellular cytotoxicity, while CHY is more sensitive than EL-4 to natural killer cell activity. Spin label probes indicate that EL-4 has a more "fluid" membrane than CHY. The density of H-2 antigen on the surface of the two lines is similar as determined by absorption of anti-H2 despite the fact that CHY is a larger cell. CHY also seems to aggregate with nonspecific CTL more extensively than EL-4.

Significance to Cancer Research: Elucidation at the molecular level of mechanisms of cell-mediated cytotoxicity may suggest reasons why some tumors are resistant to such effector function and may facilitate manipulation and modification of these processes.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $64,083

CONTRACT RESEARCH SUMMARY

Title: Diagnostic Application of Monocyte Function in Cancer

Principal Investigator: Dr. Ralph Snyderman
Performing Organization: Duke University
City and State: Durham, NC

Contract Number: NO1-CB-74121
Starting Date: 9/30/77 Expiration Date: 9/29/79

Goal: To determine the significance of abnormal monocyte function in humans with cancer.

Approach: The relationship between the clinical status of cancer patients and their monocyte chemotactic responsiveness in vitro is being determined. In addition, other parameters of phagocytic cell function are being examined to determine the specificity of depressed monocyte chemotaxis in cancer patients. The effects of serum, malignant effusions, tumor extracts, and urine from cancer patients on phagocytic cell function in vitro are being studied. The effect of tumor removal on phagocytic cell function is being examined.

Progress: The purpose of this contract is to determine the relationship of monocyte chemotactic responsiveness (MCR), tumor burden, and prognosis as well as diagnostic potential of monocyte chemotactic responsiveness in cancer patients. During the first 18 months of the contract, 167 patients with various forms of cancer have been studied. Fifty-seven of these are melanoma patients. Of these, 37 had abnormal (26 depressed and 11 elevated) MCR responses as compared to normal controls, while 20 of the 57 responded normally. The latter group had a significantly lower incidence of mortality after immunotherapy than patients with abnormal MCR. The observation that patients could have either abnormally high or abnormally low MCR responses was used as an indication that soluble factors may be present in the plasma or pleural fluid of patients which could inhibit monocyte responsiveness. Fractionation of pleural fluid from seven patients on Sephadex 6-200 has revealed the presence of a low molecular weight inhibitor of monocyte responsiveness that will be characterized further. To properly study the effects of incubating monocytes with such factor for periods up to 24 hours in vitro, a new culture technique utilizing cells in Teflon bags has been developed which maintains suitable viability for assay.

Significance to Cancer Research: The production, by neoplastic cells, of a substance which adversely affects monocyte function could be decisive in preventing the immunological elimination of cancer cells. Characterization of such a substance could have important implications with respect to both detection of cancer and therapeutic intervention.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $83,302

CONTRACT RESEARCH SUMMARY

Title: Data Management System and Statistical Support for NCI Serum Panel

Principal Investigator: Dr. Lee Richman
Performing Organization: Ebon Research Systems
City and State: Silver Spring, MD

Contract Number: NO1-CB-74210
Starting Date: 8/8/77 Expiration Date: 8/7/79

Goal: To provide data management and statistical programming support for research projects being conducted by the Committee on Cancer Immunodiagnosis in order to determine which serum tests are best able to detect early stages of cancer.

Approach: To perform statistical analyses of investigator results on NCI serum panel evaluations and to prepare summary reports of the results.

Progress: The technical team assembled by Ebon to support this effort over the past two years has proven to be highly competent, responsive, and well-suited to meet the requirements of the workscope. They have become sufficiently familiar with Immunodiagnosis Program objectives to allow them to operate relatively independently with only occasional consultation with NCI staff. During the course of the previous contract period, the Ebon staff has managed to implement data handling and statistical analysis routines that add considerable flexibility to the types of analyses that can now be performed.

Significance to Cancer Research: Since it is important to diagnose and treat cancer as early as possible, it is important to have a diagnostic and screening tool that would enable one to detect early cases of cancer. Our analyses will provide information as to the degree of success with which researchers have developed such tools.

Project Officers: Ms. Judith M. Whalen and Mr. Michael Stump
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $85,602

CONTRACT RESEARCH SUMMARY

Title: Studies of Immune Stimulants in Patients Receiving Radiation Therapy

Principal Investigator: Dr. John R. McLaren
Performing Organization: Emory University
City and State: Atlanta, GA

Contract Number: NO1-CB-64061
Starting Date: 7/16/76 Expiration Date: 6/29/80

Goal: To determine whether radiation therapy modifies the immunocompetence of patients with lung, head and neck, or cervical malignancies and to determine whether aggressive immunotherapy with combined BCG and levamisole may alter such modifications in immunocompetence and correlate with disease-free intervals or survival times.

Approach: T- and B-lymphocyte levels and percentages, in vitro lymphocyte transformation by mitogen, and skin test determinations are made on cancer patients at appropriate intervals before and following radiation therapy, and on a control population. Following radiation therapy, patients are randomized into arms with or without immunotherapy. Clinical, laboratory, X-ray, and immunological evaluations will be continued for sufficient intervals to observe disease-free intervals and survival times.

Progress: Levels of both T- and B-lymphocytes per mm^3 of blood are significantly reduced following radiation therapy, with gradual improvement shown more consistently in the group receiving no immunotherapy. Statistically significant reductions in response to both PHA and pokeweed mitogen were seen following radiation therapy in all three site groups, with a general trend toward slightly increasing mitogenic responses over time. Data suggest that the immunotherapy group shows a greater improvement following radiation therapy than does the no immunotherapy group. Responses to mumps and candida antigen skin tests remained relatively constant throughout the study period. A general trend toward increasing numbers of positive responses to streptokinase was seen in all groups. PPD responsiveness rose predictably in patients receiving BCG compared to the no immunotherapy group; however, not all immunotherapy patients responded to PPD.

Significance to Cancer Research: The data corroborate mounting evidence that cancer patients have suppressed immunocompetence, and that radiation further depresses many of these immune parameters. It is important to learn the significance of depression upon relapse and survival times.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $73,218

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of Immunodiagnostic Tests for Cancer

Principal Investigator: Dr. Adly N. Ibrahim
Performing Organization: Georgia State University
City and State: Atlanta, GA

Contract Number: NO1-CB-84267
Starting Date: 2/1/78 Expiration Date: 1/31/79

Goal: Immunodiagnosis of cervical and head and neck cancers.

Approach: Antisera are prepared in rabbits against cervical cancer tissues. Elimination of antibodies against normal tissues and normal human sera is carried out by various methods. These antisera will be used for the detection of circulating tumor-associated antigens (C-TAA) in sera of patients with cervical and head and neck cancers. Serum controls including those from normal individuals and from patients with benign diseases of these sites will be used.

Progress: The contractor has prepared hyperimmune sera against partially purified cervical cancer-associated antigens. Tumor-associated antigens were demonstrated in cervical cancer tissue and circulating tumor-associated antigens were demonstrated in the sera of patients with cervical and head and neck cancers. Testing a coded panel of sera from the NCI Mayo Serum Bank, the contractor showed significant results with high specificity but low sensitivity. No data are presented on testing of normal and benign specimens and these studies should be done. The group demonstrates an awareness of the shortcomings of their assay and offers constructive and realistic suggestions for correcting these problems.

Significance to Cancer Research: Results obtained so far suggest the possibility of developing serological tests for diagnosis, prognosis, and monitoring patients with cervical and head and neck cancers.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $75,720

CONTRACT RESEARCH SUMMARY

Title: Cells Involved in Immune Response to Tumors

Principal Investigator: Dr. Jan Cerny
Performing Organization: Harvard College
City and State: Cambridge, MA

Contract Number: NO1-CB-64035
Starting Date: 6/28/76 Expiration Date: 6/27/79

Goal: To elucidate the nature, properties, and mode of action of immunosuppressive cells (IsC) which appear in the lymphoid organs of tumor-bearing mice and which actively inhibit the functions of immunocompetent cells in the organism.

Approach: The principal experiments are done by mixing cells in vitro. Thus, cells from various organs of mice with malignant tumors are added into cultures of normal immunocompetent lymphocytes (i.e., responder cells) which are being stimulated with an antigen. A diminution of the response indicates the presence of active IsC in the given organ of the tumor-bearing donor. This system allows various manipulations of IsC, prior to their addition into responder cell cultures, in order to elucidate their functional properties.

Progress: Results prior to the present contract year showed that there are immunosuppressive cells (IsC) in the spleen of leukemic mice. These cells appear to be normal lymphocytes, distinct from leukemia cells. They are Ig-, theta-, and they suppress the immunocompetent cells via cell-to-cell contact. During the present year, we confirmed that the effect of the leukemia-associated IsC in vitro may be reversed by increasing the Ca^{++} concentration in the media; this suggests that IsC influence the cyclic nucleotide levels in the responder cells. We also found that IsC inhibit only those lymphocytes sharing K end of H-2 complex, and that they suppress the T-dependent antibody response, preferentially if not exclusively. Exciting preliminary results showed that the suppression of T-dependent response by IsC was abolished by addition of a helper factor. Our current research effort focuses on the possibility of reversing the immunosuppression in vivo.

Significance to Cancer Research: Immunosuppressive cells are now being found also in patients with tumors. It is likely that these cells represent one of the reasons why the organism is unable to mount effective immune response to the tumor as well as to various infections. The mouse model permits the analysis of this problem.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy in Outbred Cat Lymphomas and Leukemias

Principal Investigator: Dr. Myron Essex
Performing Organization: Harvard College
City and State: Cambridge, MA

Contract Number: NO1-CB-64001
Starting Date: 6/1/76 Expiration Date: 5/31/80

Goal: To see if immunotherapy using antibody to a specific tumor antigen can induce and/or sustain clinical remission in outbred cats with naturally occurring leukemia and lymphoma.

Approach: We will use specific immunotherapy, directed to the feline oncornavirus-associated cell membrane antigen (FOCMA), with and without chemotherapy. Cats with induced and naturally occurring thymic lymphoma and acute lymphoblastic leukemia will be treated with both passive and active immunotherapy.

Progress: The investigator has developed large serum pools which will be used for passive immunotherapy. All antiserum pools for therapy were titrated on both an established feline lymphoma cell culture and on FOCMA-positive FeSV transformed nonproducer mink cells that do not make the major FeLV structural proteins. The titers of antibodies to FeLV, gp70,p30, the virus neutralizing antibody titers, the levels of complement, and the levels of viremia were also determined along with the anti-FOCMA levels in the serum pools.

Eight cats were treated with anti-FOCMA serum that was heated to inactivate complement, and which did not contain antibodies to virus. Two cats were lost before the initial treatment period ended. Of the remaining 6 cats, 3 had complete remissions and 1 had a partial remission.

Significance to Cancer Research: The cat system is probably the only model where studies with spontaneous lymphoid tumors are both economically and scientifically feasible because sufficient knowledge of the tumor antigen-immune system has been developed.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $53,330

CONTRACT RESEARCH SUMMARY

Title: Tumor-specific Antigens in Diagnosis and in the Management of Cancer Patients

Principal Investigator: Dr. T. M. Chu
Performing Organization: Health Research, Inc.
City and State: Buffalo, NY

Contract Number: NO1-CB-33858
Starting Date: 6/25/73 Expiration Date: 10/24/78

Goal: To study the relationship of CEA in the evaluation of recurrence and survival for cancer patients with curative surgery.

Approach: Examine the prognostic value of the preoperative and follow-up CEA in relation to other preoperative variables; study the distribution profiles of CEA at surgery, during follow-up period, and at recurrence; implement a plan for patients whose risk of recurrence is greatest based upon preoperative and serial CEA; develop predictive models which integrate the CEA levels with other significant prognostic variables to determine the probability of recurrence in order to recommend adjuvant therapy.

Progress: An analysis of follow-up CEA data and baseline data is ongoing on 74 patients with colorectal cancer who underwent resections for cure. Data confirm that CEA rises from baseline levels at recurrence. The relationship between occurrence of follow-up CEA events to subsequent recurrence confirms the clinical observation.

Significance to Cancer Research: CEA is a laboratory assay which can be used as an aid in the management of certain cancer patients.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $71,156

CONTRACT RESEARCH SUMMARY

Title: Diagnostic Use of Leukemia-associated Antigens

Principal Investigator:	Dr. Jun Minowada
Performing Organization:	Health Research, Inc.
City and State:	Buffalo, NY
Contract Number:	NO1-CB-74165
Starting Date:	9/16/77
Expiration Date:	9/15/79

Goal: Study the expression of leukemia-associated antigens in leukemia patients at the time of disease presentation, and at appropriate intervals during and after first remission induction.

Approach: Study the expression of the following leukemia-associated antigens: non-T/non-B ALL; human thymus-leukemia antigen; and blast-leukemia-associated antigen.

Progress: A total of 450 specimens from patients with hematological malignancies were examined. A total of 240 were tested for cell surface markers which included E, EAC, SnIg, cALL antigen, T-cell antigen, and Ia-like B-cell-associated antigens. Among 14 patients with ALL, nine had non-I, non-N ALL; of two with T-cell ALL, one was positive for cALL and the other negative. Thirteen AML and three of four CML in blast crisis had an Ia-like antigen but were negative for cALL. The presence of surface antigens seems to correlate with disease activity. An attempt to overcome a serious problem of Fc binding of test reagents by nonleukemic cells was successful by a blocking procedure using heat-aggregated human IgG.

Significance for Cancer Research: Immunological subclassification provides the basis for better treatment of leukemia of an individual patient.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Adjuvant Tumor-specific Active Immunotherapy of Squamous Cell Carcinoma of the Lung

Principal Investigator: Dr. Hiroshi Takita
Performing Organization: Health Research, Inc.
City and State: Buffalo, NY

Contract Number: NO1-CB-64007
Starting Date: 6/16/76 Expiration Date: 6/15/80

Goal: To evaluate the therapeutic effects of immunotherapy with tumor vaccine given adjuvant to curative surgical resection in squamous cell carcinoma of the lung by a prospective randomized study.

Approach: Following lung resection, patients with Stage I and II squamous cell lung carcinoma are randomized into 3 groups: Group 1--control (no treatment); Group 2--specific immunotherapy; and Group 3--nonspecific immunotherapy. The specific immunotherapy group will receive solubilized squamous cell lung carcinoma antigen (500 mcg) with complete Freund's adjuvant. The nonspecific immunotherapy group will receive Freund's adjuvant only.

Progress: During the past year, 25 patients entered the study. A total of 64 patients have been accessioned, of which 58 are evaluable. Five of the 21 control patients have relapsed as have 1 of 20 treated with CFA and tumor antigen and 1 of 17 treated with CFA alone. The control patients are faring significantly worse than patients in both treatment groups. The treated groups do not differ. However, results are tentative because the number of patients in each group is relatively small and follow-up time has been relatively short.

Significance to Cancer Research: The interim results of our study indicate that adjuvant tumor-specific immunotherapy may be effective. Immunologic monitoring of the patients reveals possible effective sensitization of the immunized patients to the tumor antigen.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $93,121

CONTRACT RESEARCH SUMMARY

Title: Development of Macrophage Electrophoretic Mobility Assay for Malignant Disease

Principal Investigator: Dr. Leonard Weiss
Performing Organization: Health Research, Inc.
City and State: Buffalo, NY

Contract Number: NO1-CB-53901
Starting Date: 6/1/75 Expiration Date: 5/31/79

Goal: Investigation of the macrophage electrophoretic mobility assay and tests deriving from it as a valid tool in the diagnosis and monitoring of malignant disease.

Approach: The original MEM test is dependent on the electrokinetic response of guinea pig macrophages to lymphocyte products from patients. These macrophages are tedious to obtain, difficult to measure, and vary from animal to animal. We have therefore tested formaldehyde-fixed, tannic acid-treated sheep erythrocytes in place of macrophages in the FTEEM test.

Progress: In patients with a history of cancer, positive results were obtained in 28/42 cases with the FTEEM test compared with 32/42 in the MEM test. In people with no history of cancer, negative results were obtained in 16/18 cases with the FTEEM test, compared with 12/18 in the MEM test in the present series, and 51/69 in a more extensive series, based on a blind study organized by NCI.

Significance to Cancer Research: This assay could be potentially useful in monitoring the clinical status of patients with or with a history of cancer rather than in initial detection of the disease. It could also provide a method of examining the apparently important role played by macrophage-lymphocyte-tumor interactions in the progress of malignant disease. Depending on the medical criteria used, both the MEM and FTEEM tests give unacceptedly high levels of either false positive or false negative results for screening or general diagnostic purposes.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Adoptive Immunotherapy of Murine Leukemias Using Lymphoid Cells Sensitized In Vitro to Leukemia Antigens

Principal Investigator: Dr. Eliezer Kedar
Performing Organization: Hebrew University
City and State: Jerusalem, Israel

Contract Number: NO1-CB-64050
Starting Date: 6/30/76 Expiration Date: 6/29/79

Goal: To develop an experimental model for the immunotherapy of leukemia (and possibly of other malignancies) using conventional therapeutic modalities in conjunction with adoptive transfer of lymphoid cells sensitized in vitro to tumor cells.

Approach: Lymphoid cells from normal and tumor-bearing mice are stimulated by inactivated leukemic cells under carefully defined tissue culture conditions. The sensitized lymphoid cells are then assessed for their capacity to specifically destroy tumor cells in vitro and to eradicate leukemia implants in vivo.

Progress: In vitro sensitization of mouse lymphocytes to syngeneic and allogeneic transplantable leukemias with the generation of cytotoxic lymphocytes has been achieved. The capacity of the sensitized cells to affect tumor growth in vivo was assessed by Winn neutralization assays and by adoptive immunotherapy experiments, with or without chemotherapy in leukemia-bearing mice. A good correlation was observed between the cytotoxic activity measured in vitro (^{51}Cr release assay) and the tumor neutralizing capacity in vivo. Prolongation of survival has been obtained when the cytotoxic cells are administered 24 or 48 hours following the leukemic challenge. Survival was influenced by the source of cytotoxic cell (syngeneic cells had a greater therapeutic efficacy than allogeneic cells) and by the route of administration.

Significance to Cancer Research: This study has already demonstrated the efficacy of in vitro sensitized lymphoid cells in impeding tumor growth in vivo. It is expected that this approach could also be applied to the treatment of human malignancies.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Characterization of Antigen-binding T-cell Receptors

Principal Investigator: Dr. P. H. Krammer
Performing Organization: Institute for Immunology
City and State: Heidelberg, Germany

Contract Number: NO1-CB-74179
Starting Date: 9/30/77 Expiration Date: 9/29/79

Goal: To test for the expression of alloantigen receptors on murine T-cell tumors.

Approach: Use AKR/J lymphomas; develop sensitive assay systems for alloantigen binding T-cell receptors; obtain sufficient quantities of receptor material from positive tumors to perform biochemical analyses.

Progress: This study is designed to develop rapid screening tests for specific antigen receptors on murine T-cell tumors and then to use these tests to screen a large number of tumors. Tumors found to have specific antigen binding will be used as starting materials for biochemical analysis of receptors. Recent progress has been satisfactory. A total of 70 long-term AKR lymphoma lines have been established in vitro. A screening test which uses a fluorescent antibody sandwich technique to detect binding of target vesicles to tumor lines has been developed. Anti-idiotypic sera have been raised against responding T-cell blasts in MLR and against TNP-altered self-specific T cells and these are also available for screening receptor specificity of tumor lines.

Significance to Cancer Research: T cells play a decisive role in tumor defense. The recognition process of the T cell in general and the recognition of tumor targets in particular are not understood at all. One clue to this understanding is the biochemical nature of the T-cell antigen recognition system.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $74,100

CONTRACT RESEARCH SUMMARY

Title: Immunoprevention of Spontaneous Mammary Tumors

Principal Investigator: Dr. Etienne Y. Lasfargues
Performing Organization: Institute for Medical Research
City and State: Camden, NJ

Contract Number: NO1-CB-74201
Starting Date: 9/29/77 Expiration Date: 9/28/79

Goal: To develop immunologic means for prevention of the development of spontaneous neoplasms.

Approach: Cell surface antigens isolated by 3M KCl extraction from normal and neoplastic mouse mammary cell lines are administered by several routes to determine whether these materials will influence the development of spontaneous mammary tumors in high tumor incidence strains of mice. The effects of irradiated whole cells and of cell surface antigens from primary cultures will be compared.

Progress: The concept of the contract is to determine whether mice can be immunized with antigens from murine mammary tumors in order to reduce the incidence of spontaneous development of such tumors. To date, three protocols have been completed. In the first, 3M KCl extracts of mammary tumors originating in BALB/cfC3H mice were given intradermally, intramuscularly, or subcutaneously in different doses in an attempt to prevent the development of mammary tumors. A slight delay in tumor development was noted in experimental animals when compared to controls, but total tumor incidence at one year of age was unchanged. A second experiment using extracts of a tumor originating in C57BLfRIII to immunize animals of the same strain showed similar results. In the third protocol, extracts from a nonviral tumor line were used for immunization of BALB/c mice and, in this situation, the time of tumor appearance and tumor incidence were both unchanged as compared to controls. A fourth protocol using immunization with MMTV is currently under way.

Significance to Cancer Research: Possibility of vaccination for man.

Project Officers: Dr. Herbert Rapp and Ms. Judith Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Genetic Control of Susceptibility to Tumors

Principal Investigator: Dr. Chen K. Chai
Performing Organization: Jackson Laboratory
City and State: Bar Harbor, ME

Contract Number: NO1-CB-74181
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: Elucidate the reasons why $\underline{A^y}$ mice are more susceptible to lung tumors than $\underline{aa}$ mice.

Approach: Study the carcinogenic responses of the homozygotes and heterozygotes for the different yellow and non-yellow alleles at the agouti locus.

Progress: This contract is completing its second year of work and is incrementally funded through 1980. The effects of individual genes at the agouti (a) locus on lung tumor production are being tested in mice with C57BL/6 and C57BL/6 x A/J genetic background, using urethane to induce tumors. Preliminary results showed that $\underline{A^y a}$ mice produced more lung tumors than mice of some other genotypes (including $\underline{A^{vy} a}$, $\underline{A^{iy} a}$, $\underline{aa}$, or $\underline{a^e a^e}$. On the C57BL/J background, an average of 4.5 lung tumors in $\underline{A^y a}$ mice was found in comparison to 1.0 to 1.8 for other genotypes. The mean average for $\underline{A^y a}$ genotype on other backgrounds was also much higher than for other genotypes on that background. Since the number of mice examined so far is small, and variations within each genotype are rather large, more data will have to be obtained, but it appears that strong effects of the $\underline{A^y}$ gene on lung tumor production persist through different genetic backgrounds. The contractor has also established an $\underline{A^{iy} A^{iy}}$ line on a C57BL/6 background and produced homozygous $\underline{A^{vy}}$ mice for establishing a homozygous line on the same genetic background. The effects of yellow genes in lung tumor production can thus be tested without expression of pigment formation.

Significance to Cancer Research: To determine the role that a gene, or a specific genetic locus, plays in cancer development. The results of this investigation would specifically concern the mutation theory of neoplasm.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Preparation and Distribution of Rabbit Serum Complement

Principal Investigator: Dr. Marianna Cherry
Performing Organization: Jackson Laboratory
City and State: Bar Harbor, ME

Contract Number: NO1-CB-53870
Starting Date: 6/16/75 Expiration Date: 10/31/78

Goal: To determine whether any inbred strains of rabbits produce a serum with a consistently high level of serum complement and, if so, to investigate ways of making this reagent available to the research community for testing normal and malignant cells.

Approach: To screen sera from 10 rabbits of each sex from each of 10 inbred or partially inbred rabbit strains in our colony by complement-dependent cytotoxic assays, first with mouse target cells (normal and malignant) and then, in cooperation with other laboratories, with human cells of normal or malignant origin.

Progress: Complement-dependent cytotoxic assays are widely used in studies of human cells, both normal and malignant, for identification of antigens of the cell membrane, particularly those specific for malignant cells. Complement, a collective group of serum proteins, must be both high in specific activity and low in nonspecific toxicity for the cells to be tested. By tests with normal and malignant cells from mice, it was shown that certain inbred rabbit strains consistently produce higher quality complement than others. Serum samples from 19 strains of rabbits and 2 hybrids have been studied, and samples have been sent to other laboratories for independent screening.

Significance to Cancer Research: Alteration of cell membrane antigens generally accompanies transformation from normal to neoplastic cells. Studies of these antigens are difficult technically, the more so due to the lack of a reliable source of complement. If certain inbred rabbit strains do, as we believe, provide high specific complement levels, the many ongoing projects in this area should be materially assisted.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: The Development and Maintenance of New Congenic Mouse Strains

Principal Investigator: Dr. Edwin D. Murphy
Performing Organization: Jackson Laboratory
City and State: Bar Harbor, ME

Contract Number: NO1-CB-74174
Starting Date: 9/30/77 Expiration Date: 9/29/79

Goal: Develop and maintain new congenic mouse strains as models for determining basic mechanisms of lymphoproliferation and autoimmunity and their role in tumorigenesis.

Approach: Isolation of genetic factors controlling lymphoproliferation and autoimmunity and development of congenic models by selection and backcrossing for mutant gene lpr and establishing reciprocal F_1 hybrids for strain BXSB. Production of basic models for distribution.

Progress: The discovery of a mutant gene, lpr (lymphoproliferation), controlling massive T-cell proliferation and autoimmunity opened the way for the development under this new contract of the first congenic inbred strains that permit fully controlled studies of the effects of spontaneous autoimmunity on tumorigenesis. The transfer of lpr to reference strain MRL-+/+ has now reached the 6th backcross generation (N6), establishing highly congenic inbred strain MRL-lpr/lpr with estimated residual heterozygosity <0.4%. During the period of development and breeding colony expansion, it has been possible to make available for preliminary study by other investigators limited numbers of the original models, strains MRL/l, MRL/n, and BXSB. Transfer of lpr to strains of major importance in cancer research is now at the backcross generation indicated in parentheses: A/HeJ (3), BALB/cJ (4), C3H/HeJ (3), C57BL/6J (6), C58/J (3), NZB/BlNJ (4), and SJL/J (6). A second model for lymphoproliferation and autoimmunity, recombinant inbred strain BXSB, shows inheritance as an autosomal dominant trait with acceleration of the disease in F_1 hybrid males produced by a factor contributed by the BXSB male parent.

Significance to Cancer Research: These congenic models open the way to precisely controlled study at the molecular and cellular levels of the interaction of genes, viruses, and sex-related factors in autoimmunity and regulation of lymphocyte subset proliferation, and the role of autoimmunity and the immune processes in tumorigenesis.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Immune Assays for Enzymes and Isozymes in Cancer

Principal Investigator: Dr. Joel Shaper
Performing Organization: Johns Hopkins University
City and State: Baltimore, MD

Contract Number: NO1-CB-84260
Starting Date: 9/22/78 Expiration Date: 9/21/79

Goal: Development of immunodiagnostic assays for cancer using an enzyme or isozyme as an antigen and looking for a correlation of enzyme/isozyme serum levels with tumor burden.

Approach: Evaluate the immunodiagnostic significance of serum levels of UDP-galactosyltransferase in human breast and ovarian carcinoma.

Progress: Antisera directed against affinity-purified bovine UDP-galactosyltransferase have been prepared in rabbits and partially immunologically characterized. These antisera have relatively high titered precipitating antibody to bovine galactosyltransferase as assessed by Ouchterlony double diffusion technique. The antisera inhibit galactosyltransferase enzyme activity and finally the antisera are highly cross-reactive with human UDP-galactosyltransferase found in both normal serum and ascitic fluid obtained from a patient with ovarian cancer. This cross-reactivity will form the experimental basis for the development of a sensitive specific radioimmunoassay for human serum UDP-galactosyltransferase using the purified bovine enzyme as antigen.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $65,562

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of Immunodiagnostic Tests for Cancer

Principal Investigator: Dr. John Fenimore Cooper
Performing Organization: Kaiser Foundation
City and State: Oakland, CA

Contract Number: NO1-CB-74169
Starting Date: 9/16/77 Expiration Date: 9/15/79

Goal: Evaluate an immunodiagnostic test for prostatic cancer.

Approach: Accumulate and assess clinical oncologic data for prostatic cancer with a solid phase radioimmunoassay technique for prostatic acid phosphatase (RIA-PAP); continue development of the RIA-PAP technique as a screening technique in the early diagnosis of prostatic cancer utilizing a large random population of elderly males.

Progress: It appears that in the last year, 2,000 randomly selected males over the age of 45 have been screened using a radioimmunoassay for acid phosphatase. The total number screened in 16 months is 6,300 and, of these, 350 had abnormal levels above 6.5 ng/0.1 ml of serum. Of these, approximately 25% have been diagnosed as having prostatic cancer, 40% are still suspect, and 20% remain normal.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $40,444

CONTRACT RESEARCH SUMMARY

Title: Diagnostic Applications of Antibodies to Melanoma

Principal Investigator: Dr. Peter Hersey
Performing Organization: Kanematsu Institute
City and State: Sydney, Australia

Contract Number: NO1-CB-74120
Starting Date: 9/30/77 Expiration Date: 9/29/79

Goal: Determine whether there is a correlation between the clinical course of melanoma growth and levels of serum antibodies.

Approach: Employ lymphocyte-dependent cytotoxic antibody assays.

Progress: The workscope of this contract is to determine whether there is a correlation between the clinical course of melanoma growth and levels of serum antibodies using leukocyte-dependent cytotoxicity antibody (LDA) assays. During the first 18 months of this study, 125 patients have been entered. Of these, 67 had LDA activity detectable against heterologous cultured melanoma cells. In those patients showing LDA activity against non-melanoma cells, absorption with fetal tissue removed the activity from sera. In 45 patients, a rise in LDA activity immediately after surgery was seen with a subsequent decay. Four multiparous women with melanoma showed high LDA activity throughout their disease course. In 18 of 76 patients not showing LDA reactivity, fractionation of their serum to purify the immunoglobulin fraction resulted in "unblocking" and measurable LDA activity. The period of follow-up is presently too short to permit correlations between lymphocyte-dependent antibody-mediated lysis and the clinical course of melanoma.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $49,381

CONTRACT RESEARCH SUMMARY

Title: Cells Involved in Immune Response to Tumors

Principal Investigator: Dr. Eva Klein
Performing Organization: Karolinska Institute
City and State: Stockholm, Sweden

Contract Number: NO1-CB-64023
Starting Date: 6/28/76 Expiration Date: 6/27/79

Goal: Characterizing the naturally occurring cytotoxic cells (NK cells) in mice and man, partly to assess their significance and partly to develop methods to remove them, in order to reveal possibly specific reactions that are otherwise masked by them.

Approach: Lymphocyte fractionation and lymphocytotoxicity in human systems; the same in combination with in vivo rejection tests in mouse systems.

Progress: The insensitivity of some highly NK cell sensitive Moloney lymphomas to T-cell killing was shown to be an inherent membrane property of the target cells and not due to the absence of the potentially insensitive target site. A difference was found between the ability of in vivo maintained YAC lymphoma and its in vitro subline to generate cytotoxic T-sites in vitro, perhaps related to the ability of the in vivo YAC tumor to generate suppressor cells that counteracted the priming effect of syngeneic but not of allogeneic tumors. A close parallelism was found between the hybrid resistance against bone marrow grafts and NK cell-mediated resistance. NK activity of radiochimeric animals corresponded to the bone marrow donor, not the recipient host. Similar findings were made on "age" chimeras. Murine and human NK cells had a certain cross reactivity, but with a better fit for targets of the corresponding species. Genetic patterns of resistance were identified in relation to the NK-mediated lysis and in vivo F_1 hybrid resistance (presumably NK-mediated) against a variety of tumors.

Significance to Cancer Research: It is essential at this time to distinguish between nonspecific and specific killer effects in both mouse and human tumor systems and to initiate studies on the possible in vivo relevance of in vitro lymphocytotoxicity.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: In Vitro Immunization with Autochthonous Human Tumor

Principal Investigator: Dr. Eva Klein
Performing Organization: Karolinska Institute
City and State: Stockholm, Sweden

Contract Number: NO1-CB-74144
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: To demonstrate the existence of specific human tumor-associated antigens and to show in vitro increased responses to such antigens after in vitro immunization with autochthonous tumor.

Approach: Human tumor specimens (carcinoma of the lung, astrocytomas, osteo- and soft tissue sarcomas) will be characterized and fractionated for tumor and lymphoid cells. Cytotoxicity tests will be performed with biopsy target cells using blood lymphocytes and tumor infiltrating lymphocytes, as effectors, both directly at harvest and after cocultivation in vitro. Attempts will be made to characterize the nature of the effector cell.

Progress: Proliferative and cytotoxic responses of peripheral blood lymphocytes were studied against autologous tumor cells. Blastogenesis to autologous tumor cells was observed in most of the patients studied, whereas the response of those cells to alloantigens was observed much less frequently. Direct cytotoxic effects against autochthonous tumor cells were observed using lymphocytes from 11 to 15 patients. Killing against allogeneic targets was infrequently seen. Lymphocytes from healthy donors showed little or no cytotoxicity.

The proliferative and cytotoxic responses of tumor-infiltrating lymphocytes to tumor are also being studied.

Significance to Cancer Research: The question whether the human tumor patient reacts against and/or can be sensitized against his autologous tumor is the central problem of human tumor immunology.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $75,173

CONTRACT RESEARCH SUMMARY

Title: Role of the Macrophage in Tumor Resistance

Principal Investigator: Dr. D. S. Nelson
Performing Organization: Kolling Institute
City and State: St. Leonards, Australia

Contract Number: NO1-CB-63973
Starting Date: 7/16/75 Expiration Date: 10/31/78

Goal: To determine what roles macrophages may play in resistance to tumors and whether they can be exploited in immunotherapeutic strategies.

Approach: The growth of grafts of chemically induced tumors is measured in the feet of mice. Studies are made of the effects of various agents and procedures on the growth rate of tumors in normal and immune mice. The mice are immune either (a) during carriage of a tumor (concomitant immunity, at first specific and later nonspecific) or (b) after injection of a small number of tumor cells (sinecomitant immunity, always specific). In parallel, the effects of macrophages and other cells on the survival and multiplication of cultured tumor cells are measured.

Progress: Tumor rejection could be initiated by T cells with Ly markers (1^+, 2^-, 3^-) characteristic of T cells initiating DTH reactions. Intraperitoneal (i.p.) challenge of mice with the tumor to which they were immune specifically triggered influx into the peritoneal cavity of cells with the morphology of macrophages. Further investigation of anti-tumor effector cells has shown that they occur not only in the peritoneal cavities of tumor-immune mice, but also in peritoneal exudates induced in normal mice by proteose peptone, or following infection with Listeria, Salmonella or BCG. The cells were separated on the basis of sedimentation velocity. Large macrophages had an entirely nonspecific cytostatic effect. Smaller noncytoid cells had a tumor-selective, but not individual tumor-specific, slow cytotoxic effect, different from the effect of NK cells on susceptible targets. Specific i.p. challenge of tumor immune mice led to a selective increase in cells of the latter type.

Significance to Cancer Research: Immunity to the tumors studied involves increased production of naturally occurring nonspecific effector cells (macrophages) and the development of a specific delivery mechanism, akin to delayed-type hypersensitivity, which overcomes the antagonistic effects of tumor cell products.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Characterization of Factors Causing Inhibition of Macrophage Function or Inflammatory Responses

Principal Investigator: Dr. D. S. Nelson
Performing Organization: Kolling Institute
City and State: St. Leonards, Australia

Contract Number: NO1-CB-84251
Starting Date: 9/30/78 Expiration Date: 9/29/80

Goal: To conduct research designed to functionally characterize factors produced by and/or induced by tumors, which impair macrophage function and/or inflammatory responses in vivo and in vitro.

Approach: Attempts will be made to evaluate the nature, extent, mode of action, and duration of impairment of macrophage function and inflammatory responses produced directly or indirectly by tumor cell products. Tumors will be of mouse, rat, and human origin. The effects of such factors on monocytes, stimulated and activated macrophages, and on tumor-associated macrophages will be compared. In addition, studies will be undertaken to assess the significance of such factors with respect to tumor growth in animals.

Progress: Several technical problems in the production, storage, and fractionation of active factors in tumor cell culture supernatants (TCCSs) have been identified and, in part, solved. Partial purification of low and high M.W. factors has been achieved.

Studies are being conducted to assess the effects of these factors on (1) delayed-type hypersensitivity reactions of mice to sheep erythrocytes; (2) the cellular composition of peritoneal fluid following intraperitoneal injection of active factors; (3) the inhibition of macrophage phagocytosis in vitro; and (4) nonspecific cytotoxicity of activated macrophages to tumor cells in vitro.

Significance to Cancer Research: There is increasing evidence that macrophages may have an important effector function to play in cell-mediated immunity both to infectious agents and tumors. Thus, an understanding of factors impairing macrophage function are of interest in the consideration of immunotherapeutic approaches to cancer.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $57,409

CONTRACT RESEARCH SUMMARY

Title: Diagnostic Application of Human Tumor-associated Antigens

Principal Investigator: Dr. Calvin A. Saravis
Performing Organization: Mallory Institute of Pathology
City and State: Boston, MA

Contract Number: NO1-CB-64071
Starting Date: 6/16/76 Expiration Date: 5/31/79

Goal: To develop an assay system for the tumor marker, ZGM, that is useful in the diagnosis and management of digestive tract malignancy.

Approach: To further refine a radioimmunoassay for detecting ZGM in the blood of patients with gastrointestinal cancer and make a clinical evaluation of it by comparing it to CEA.

Progress: Avid high titer anti-ZGM antisera were produced to purified ZGM and used to analyze tumor and normal tissue extracts. ZGM was detected in primary and metastatic colon cancer and was not detected in normal human plasma, normal colon, normal liver, and normal spleen. It was distinct from CEA, from antigens cross-reacting with CEA, AFP, alpha-2 macroglobulin, ferritin-like molecules, blood group antigens, and buffy coat. ZGM showed no reaction in a radioimmunoassay for CEA. During the present contract year a ZGM radioimmunoassay was developed. The ZGM-RIA measured less than 2.5 ng of ZGM standard. RIA analysis of both ZGM and CEA activity in the same plasma specimens obtained from patients with benign diseases, large bowel polyps, colonic malignancy, and noncolonic malignancy gave different and complementary findings.

Significance to Cancer Research: The detection of the ZGM in patient specimens may permit earlier diagnosis, better evaluation of prognosis, and monitoring of therapy for cancer.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Phase I Study of Effects of Immune Stimulants on Human Immune Responses

Principal Investigator: Dr. Michael J. O'Connell
Performing Organization: Mayo Foundation
City and State: Rochester, MN

Contract Number: NO1-CB-53874
Starting Date: 6/1/75 Expiration Date: 11/31/78

Goal: To evaluate the effects on immunologic determinants, therapeutic effects, and toxicity of BCG-Tice strain (lyophilized) versus BCG-Pasteur strain (lyophilized) versus BCG-Pasteur strain (fresh frozen) in patients with advanced cancer.

Approach: Patients, stratified by performance status and reactivity to recall antigen skin tests, are randomized on a double blind basis to one of the three BCG preparations or to a placebo. Treatments are given by the tine technique on either weekly or monthly schedules, depending upon distance from the Mayo Foundation. Serial assessments of several immune parameters are made.

Progress: Local cutaneous toxicity resulting from the BCG treatments was mild to moderate with only three instances of ulcerative skin toxicity observed. Febrile reactions were roughly equal in the BCG and placebo groups; however, liver function abnormalities were seen significantly more frequently in patients receiving BCG therapy compared to placebo. No cases of disseminated BCG infection, fatalities ascribed to BCG therapy, or other evidence of toxic reactions could be identified. There was no evidence that improvements in immune status occurred more frequently in patients receiving any of the BCG preparations regardless of schedule as compared to placebo. None of the patients with measurable malignant disease experienced an objective tumor response regardless of treatment given. Survival was not improved in any of the BCG patient groups as compared to patients receiving placebo.

Significance to Cancer Research: The value of using the patient with advanced refractory cancer as a model for the study of immune stimulants will be determined. Identification of a more potent and/or less toxic form of BCG preparation would have practical clinical applications to future clinical trials involving BCG immunotherapy.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Maintenance of the NCI Serum Bank

Principal Investigator: Dr. Allan J. Schutt
Performing Organization: Mayo Foundation
City and State: Rochester, MN

Contract Number: NO1-CB-84258
Starting Date: 9/30/78 Expiration Date: 11/30/80

Goal: To establish and maintain a bank of sera from patients with cancer, with benign diseases and from normal individuals.

Approach: Make necessary serum samples available for evaluation of immunodiagnostic tests for cancer. Serve as a central facility for storage of serum and plasma specimens collected by other contractors in the Tumor Immunology-Immunodiagnosis Program.

Progress: New contract.

Significance to Cancer Research: This serum bank will provide sera, in sufficiently large quantities and with adequate controls, to efficiently and rapidly evaluate potential immunodiagnostic tests for cancer.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Diagnostic Use of Cross-reacting Microbial Antigens

Principal Investigator: Dr. Christine H. Granatek
Performing Organization: M.D. Anderson Hospital
City and State: Houston, TX

Contract Number: NO1-CB-74122
Starting Date: 9/16/77 Expiration Date: 9/15/79

Goal: To study the distribution of an isolated microbial cross-reactive antigen in human leukemia and melanoma, produce specific antisera, and develop a radioimmunoassay for detection of circulating cross-reactive antigen.

Approach: Initial screening of tumor cells by immunofluorescence and of cancer patients' sera by a solid phase RIA, to detect reactivity with antimicrobial sera. Isolation of cross-reactive components by affinity chromatography. Specificity testing by absorption studies and cross-testing in RIA, with normal donor and benign disease patient control.

Progress: This project is in its second year. Recent emphasis has been placed on phase two of the project, preparation of purified BCG cross-reactive antigens and antisera for the development of a radioimmunoassay for pilot clinical immunodiagnostic tests. The primary tumor-associated BCG cross-reactive component isolated by affinity chromatography of AML and melanoma extracts was a glycoprotein of approximately 70,000 molecular weight which also had fetal cross-reactivity. This component has been purified on an antifetal affinity chromatography column and monospecific antibody has been produced against antigen isolated from a mouse fetal liver source. This component has widespread antigenicity in leukemia and melanoma patients, and a radioimmunoassay is being developed to test these patient's sera for the circulating antigen. A preliminary clinical study has suggested the prognostic utility of measuring AML patients' antibody levels to BCG-CWS (cell wall skeleton) or BCG cross-reactive antigen as well as to a leukemia-associated antigen in preparation.

Significance to Cancer Research: Assays based on either detection of circulating antigen or antibody may be useful in clinical diagnosis or monitoring of patients with these malignancies.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $88,005

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy of Disseminated Human Cancer

Principal Investigator: Dr. Evan Hersh
Performing Organization: M.D. Anderson Hospital
City and State: Houston, TX

Contract Number: NO1-CB-33888
Starting Date: 6/25/73 Expiration Date: 7/7/80

Goal: To develop immunological treatments for patients with human cancer.

Approach: Patients receive phase I and phase II immunotherapy agents including BCG, C. parvum, MER, Pseudomonas vaccine, levamisole, thymosin, and purified tumor antigen. These fall into the approaches of active-nonspecific, active-specific, and immunorestorative immunotherapy. Patients are treated either after surgery when they have no evident disease, or in combination with chemotherapy for potential or actual metastatic disease.

Progress: Sufficient numbers of patients have entered the study of chemo-immunotherapy of malignant melanoma with or without i.v. C. parvum, DIC plus actinomycin to determine that the response rate is about 20-25% and that the addition of C. parvum will not make a significant contribution to remission duration and survival. Patient entry is continuing in a study of patients with Stage II and III breast cancer who are being treated with chemotherapy with 5-FU, adriamycin, and cyclophosphamide with or without BCG immunotherapy. Patient entry is continuing on a study of patients with AML over the age of 50 who are randomly assigned to ROAP chemotherapy with and without MER; on a Phase I study of thymosin; and on a Phase I study of BCG CWS plus P2 plus endotoxin.

Significance to Cancer Research: It is important to confirm in prospectively randomized trials earlier studies showing immunotherapy to be of benefit to cancer patients.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $258,500

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy in the L_2C Guinea Pig Leukemia

Principal Investigator: Dr. Samuel G. Murphy
Performing Organization: M.D. Anderson Hospital
City and State: Houston, TX

Contract Number: NO1-CB-64003
Starting Date: 6/30/76 Expiration Date: 12/31/78

Goal: To evaluate dose, route, and schedule of administration of immunotherapeutic agents and to investigate the structuring of sequential and combination immunotherapeutic agents in the L_2C guinea pig leukemia model.

Approach: Animals with leukemia are treated with systemic chemotherapy and subsequent radiation therapy to the cranial spinal axis to prevent central nervous system leukemia. During the period of complete remission, animals are randomized to experimental groups and treated with immunotherapeutic agents. Two variables such as route and schedule of administration are held constant and dosage is varied in the experimental groups. Animals are monitored by peripheral blood counts to determine the duration of remission.

Progress: Studies have been undertaken to evaluate the efficacy of several immunotherapeutic agents in the treatment of leukemic guinea pigs which have also received BCNU chemotherapy and irradiation. Earlier results showing some immunotherapeutic benefit from C. parvum could not be confirmed in subsequent experiments. Investigators have also evaluated BCG by several doses and routes of administration. A trend toward a positive effect was noted in preliminary studies with intracardiac BCG.

Significance to Cancer Research: This animal model was developed to investigate the interaction of specific and nonspecific immunotherapy in an animal model whose pathophysiology and therapy of disease closely resembled human acute leukemia. This model should be helpful in establishing biological variables that influence the effectiveness of immunotherapeutic agents.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Collection of Serial Serum Samples from Cancer Patients

Principal Investigator: Dr. Carl Feit
Performing Organization: Memorial Hospital
City and State: New York, NY

Contract Number: NO1-CB-74124
Starting Date: 9/30/77 Expiration Date: 9/29/79

Goal: Collect serial serum specimens from cancer patients and benign disease controls for evaluation of potential immunodiagnostic tests for cancer.

Approach: Collect serial specimens from patients with melanoma, ovarian carcinoma, lung carcinoma, and Hodgkin's disease, plus suitably matched patients with benign skin lesions, benign adnexal masses, a history of heavy smoking, and benign lymphadenopathy to serve as controls.

Progress: This contract is completing the second year of a projected five-year project for collection of serial serum specimens from cancer and control patients for use in evaluating potential immunodiagnostic tests for cancer. Patient entry has been under way for a year and an average of 16 patients per month have been admitted to the study. Nearly 5,000 ampules of serum have been collected and stored, and extensive clinical information has been logged in. It is projected that entry of patients into the study should be completed by late 1980; then two additional years of follow-up specimen collection are required. A statistician has been consulted to determine whether an increase in frequency of sample collection or size would be desirable to get the most benefit from the contracted work.

Significance to Cancer Research: The effective evaluation of the prognostic value of immunodiagnostic tests requires testing sera from patients and controls which are well matched and have been collected in a serial prospective fashion. Establishing collections of such sera will expedite the evaluation of new immunodiagnostic assays as they become available.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $93,919

CONTRACT RESEARCH SUMMARY

Title: Studies on Macrophage Activation

Principal Investigator: Dr. Emil Skamene
Performing Organization: Montreal General Hospital
City and State: Montreal, Canada

Contract Number: N01-CB-84269
Starting Date: 9/30/78 Expiration Date: 9/29/79

Goal: Study the interactions of T cells for their products with macrophages that lead to activation of macrophages for reaction against tumor cells.

Approach: Investigate the identity and function of cell populations that control macrophage activation in the development of antilistereal resistance and determine the cellular level of phenotypic expression of genetically controlled high or low levels of antilistereal immunity.

Progress: During the first four months of this contract, the investigators have concentrated on that portion of the workscope aimed at elucidating the genetics of Listeria sensitivity and resistance in appropriate mouse strains. By the use of various breeding protocols, they have developed evidence that a single codominant trait which affects the differentiation of a bone marrow-derived macrophage precursor is involved. The strains such as A/J which are sensitive to Listeria infection show a block in the development of this precursor to a mature macrophage capable of conferring a protective immune response. Future work will continue the genetic studies in congenic mouse strains as well as investigate the T cell-macrophage interactions which lead to macrophage activation in resistant strains such as B.6 and B10.A.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $96,400

CONTRACT RESEARCH SUMMARY

Title: Chemoimmunotherapy of Acute Myelocytic Leukemia

Principal Investigator: Dr. James F. Holland
Performing Organization: Mt. Sinai School of Medicine
City and State: New York, NY

Contract Number: NO1-CB-43879
Starting Date: 6/30/74 Expiration Date: 6/20/80

Goal: To evaluate the usefulness of neuraminidase-treated allogeneic AML cells (VCN-AML) in acute myelocytic leukemia (AML).

Approach: Following remission induction with cytosine, arabinoside, and daunorubicin, AML patients had earlier been randomly assigned to maintenance chemotherapy alone, chemotherapy in combination with VCN-AML or in combination with VCN-AML plus MER. The MER arm has been deleted from the protocol. Patients are studied with both in vivo and in vitro immunologic tests.

Progress: Statistical analysis of results on 114 patients conclude that there is a significantly better experience in the group getting chemotherapy plus neuraminidase-treated allogeneic AML cells as compared to control groups, based on an unadjusted analysis (i.e., without taking into account other potential prognostic variables). Lymphocytes from 11 control and 18 patients were tested for proliferative response to autochthonous myeloblasts. Results suggest remission lymphocytes from patients receiving chemotherapy alone do not react against the autologous monoblasts, while increased proliferative activity was observed when remission lymphocytes from patients who received neuraminidase-treated myeloblasts were exposed to their myeloblasts in vitro.

Significance to Cancer Research: Preliminary data analysis suggests that the combination of sustaining chemotherapy with neuraminidase-treated allogeneic AML cells may represent a significant advance in the treatment of AML.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $209,910

CONTRACT RESEARCH SUMMARY

Title: Cryopreservation of Human Monocytes

Principal Investigator: Dr. J. A. Loos
Performing Organization: Netherlands Red Cross
City and State: Amsterdam, The Netherlands

Contract Number: NO1-CB-74164
Starting Date: 9/30/77 Expiration Date: 9/29/79

Goal: Find the optimal conditions for cryopreservation of human monocytes.

Approach: Analyze the following variables: purification methods, concentration of cryoprotectant, controlled cooling rate, the thawing procedure, and dilution and subsequent removal of the cryoprotectant. Once optimal cryopreservation is achieved, functional studies will be done.

Progress: Work on cryopreservation of monocytes has continued with the adherence method having a high yield but also a high percentage of dead cells and the centrifuge method having a lower yield but very low percentage of dead cells. With either method 80% purity is obtained. The following parameters are tested before and after freezing and thawing: recovery, oxygen consumption during phagocytosis, cytotoxicity against anti-D-sensitized red cells, chemotaxis toward casein, killing of S. aureus, and helper function in mitogenic response to Con A. The following freezing conditions were varied: cell medium composition, concentration of cryoprotectant (DMSO), cell concentration, ampule volume, and cooling rate. The contractor's studies have led him to conclude that human monocytes can be frozen for functional preservation.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $54,300

CONTRACT RESEARCH SUMMARY

Title: Animal Models for Bone Marrow Transplantation

Principal Investigator: Dr. Ross S. Basch
Performing Organization: New York University Medical Center
City and State: New York, NY

Contract Number: NO1-CB-74176
Starting Date: 9/30/77 Expiration Date: 9/29/79

Goal: To develop an animal model for transplantation of selected bone marrow cells of defined function and developmental potential.

Approach: To study the relationship between the hematopoietic precursors of immunocompetent T cells and the "natural killer cells" of bone marrow and spleen in mice.

Progress: This project is completing its second year. It appears that at least some NK activity is produced by cells of the T-cell lineage which, rather than being "pre-thymic," represents an alternative pathway in the development of thymocytes. It has been necessary to spend a lot of time on solving problems surrounding quantitation of NK activity. While progress has been made, some problems still exist. NK activity is not directly related to the number of input cells. Both the scope of the curve and the magnitude of the ^{51}Cr release vary among the strains tested when assayed. Another problem encountered is that the same number of cells from the same group of animals, tested against the same targets, can mediate different amounts of killing. Therefore, NK activity is the product of the number of NK cells and the activity of each cell. Since different serum preparations differ in their effect on NK activity and ag-ab complexes appear to have significant effect on the adherent cells which seem to regulate NK activity, the assessment of the activity of a preparation subject to a variety of serologic tests is difficult. It also appears as if all NK activity is not the product of identical cells. Injection of mice with BCG produces significant increases in NK activity measured 2-6 weeks post injection. The NK activity of BCG-treated mice has been less sensitive to thymopoietin inhibition and more sensitive to anti-mouse brain serum than that of normal mice. If different cell types are involved and all results are a composite of the activities of these cells, quantification becomes extremely difficult.

Significance to Cancer Research: If natural resistance to malignancy is mediated by an immature member of the T-cell lineage, it may be possible to influence the rate of passage of cells through this compartment and alternately influence the magnitude of natural response.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $66,340

CONTRACT RESEARCH SUMMARY

Title: Clinical Application of Assays for Tumor-associated Antigens

Principal Investigator: Dr. Clement C. S. Hsu
Performing Organization: Northwestern University
City and State: Evanston, IL

Contract Number: NO1-CB-74132
Starting Date: 9/26/77 Expiration Date: 9/25/79

Goal: To investigate the clinical applicability of rabbit antisera, anti-EP and anti-ALL, prepared in our laboratory, and the clinical relevance of cell-bound blocking component on leukemic lymphocytes.

Approach: By serial examination of lymphocytes from patients with lymphocytic leukemias during the course of illness and patients with other diseases for the anti-EP and anti-ALL reactivity.

Progress: Marrow cells or blood lymphocytes from 103 ALL, 37 CLL patients, and 123 other individuals have been studied so far. Approximately 80% of the ALL cases were null-ALL without sheep erythrocyte receptor, a T-lymphocyte marker, or B-cell marker on leukemic cells. Two antisera to human leukemic cells have been prepared, one reacting with B cell-associated antigens (designated NBAA) on leukemic lymphocytes and normal B cells, and the other with an ALL cell-specific antigen (designated ALLA). Preliminary clinical follow-up indicates that ALLA(-) cases had a poorer prognosis than did ALLA(+) cases. ALL plasmas were shown to inhibit anti-ALL reactivity with ALL cells. An efficient method of eliminating nonspecific fluorescent stain in monocytes or myeloid cells has been devised which greatly facilitates detection of ALLA(+) cells in blood or marrow. An antibody prelabeling procedure has been set up to examine in vitro the rate of disappearance by shedding and endocytosis, and regeneration of lymphocyte surface antigens.

Significance to Cancer Research: Our results indicate the usefulness of anti-ALL in differential diagnosis and subclassification of ALL. Further studies of cell-bound blocking component may lead to understanding of another mechanism by which leukemic cells evade recognition and destruction by host immunological apparatus.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $70,611

CONTRACT RESEARCH SUMMARY

Title: Antigenicity of Precancerous Lesions in Animal Models

Principal Investigator: Dr. Richard G. Olsen
Performing Organization: Ohio State University
City and State: Columbus, OH

Contract Number: NO1-CB-74159
Starting Date: 9/30/77 Expiration Date: 9/29/79

Goal: To determine whether precancerous cells carry antigenic markers which are distinguishable either in type or concentration from those exhibited by normal or by malignant cells.

Approach: Using the Rickard strain of feline leukemia virus (FeLV) in SPF cats, study will be made of the sequential development of feline oncornavirus associated cell membrane antigen (FOCMA) in preleukemic bone marrow cells in cats infected with FeLV. Correlations will be sought with other markers associated with the preleukemic phase of the disease.

Progress: The cell membrane antigen (FOCMA) bound to tumor cells of kittens infected with feline leukemia virus has been demonstrated to be present on bone marrow cells three weeks after virus infection and on mesenteric lymph node cells four weeks after virus infection. Studies are under way to determine whether FOCMA antigen is a useful marker for preneoplasia.

Significance to Cancer Research: The immunologic rejection of tumor tissue is recognized as the pivotal factor in the control of cancer. These studies will determine whether qualitative and/or quantitative differences in tumor antigen expression on the early preneoplastic lesion influence the subsequent course of tumor development.

Project Officers: Dr. Herbert Rapp and Ms. Judith Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $78,925

CONTRACT RESEARCH SUMMARY

Title: Animal Models for Treatment of Minimal Residual Systemic Tumor

Principal Investigator: Dr. Gerald L. Bartlett
Performing Organization: Pennsylvania State University
City and State: Hershey, PA

Contract Number: NO1-CB-33891
Starting Date: 6/30/73 Expiration Date: 10/31/79

Goal: To develop animal tumor models which will be useful for evaluating immune stimulants as immunotherapeutic agents.

Approach: In four different tumor-host models, animals are given immunotherapy at various times after tumor implantation, either alone or in combination with other means of treatment. The effect of the immunotherapy and the role of immune stimulants in immunotherapy are evaluated in terms of improved survival of treated animals. Animals which survive are tested further for improved tumor rejection immunity.

Progress: The investigators have evaluated several immunotherapeutic agents in four animal tumor models. (1) In the treatment of minimal s.c. tumor of the CaD2 mammary adenocarcinoma, the relative therapeutic efficacy of the agents studied was C. parvum, BCG>B. pertussis; LMS was ineffective. (2) In the prevention and treatment of 13762 rat mammary adenocarcinoma, many agents were studied including live and irradiated BCG, Nocardia rubra CWS, C. parvum, MDP, inactivated tumor cells. (3) In the line 10 guinea pig hepatoma, optimal conditions have been determined for the growth of line 10 cells in vitro. B. pertussis had some adjuvant activity in the L10x vaccine. (4) In the LSTRA mouse leukemia model C. parvum was an effective immunoprophylactic and therapeutic agent. Lx vaccines were able to prolong survival or cure mice treated two days after tumor challenge. This effect was potentiated by C. parvum. Killed BCG, viable BCG, or BCG CW-OD were ineffective adjuvants for the Lx vaccine. Although there were some favorable results, the variability of results emphasized the hazard of drawing "generalizations" from results in an experimental setting.

Significance to Cancer Research: These studies demonstrate that caution must be exercised in drawing generalizations on immunotherapy from a single experimental or clinical result.

Program Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group:: Tumor Immunology Committee
FY 79 Funds: $231,492

CONTRACT RESEARCH SUMMARY

Title: Immunogenicity of "Spontaneous" Animal Tumors

Principal Investigator: Dr. Gerald L. Bartlett
Performing Organization: Pennsylvania State University
City and State: Hershey, PA

Contract Number: NO1-CB-64039
Starting Date: 6/16/76 Expiration Date: 6/15/79

Goal: To test whether "spontaneous" murine tumors appear to be nonantigenic due to processes which are known to interfere with cell-mediated cytotoxicity.

Approach: Obtain new tumors likely to be nonimmunogenic ("spontaneous" or induced by means not associated with strong tumor antigens). Test immunogenicity of the tumors in sensitive in vivo tests (immunization-challenge, Winn assay). Determine the effect on immunogenicity of procedures intended to: (1) deplete suppressor activity; (2) remove blocking factors; or (3) "reactivate" sensitized cells.

Progress: Nine spontaneous and 12 induced tumors were established by passage in syngeneic mice and are stored in liquid nitrogen. Nine other recently induced tumors were studied to define their biological and immunological properties. So far any tumors nonantigenic in immunization challenge tests were also nonantigenic in the Winn test. Incorporation of BCG as an adjuvant in immunizing tumor cell suspensions did not improve host response to the tumor. Pretreatment of the mice with cyclophosphamide and/or thymectomy did not cause better immunity to these tumors. Overall on this contract, biological characterization is complete for each of the tumors selected for study; immunogenicity data are available for most of them and will soon be available for the last three (of 11). This tumor panel was selected as the number of tumors became unwieldy and it is expected that immunogenicity and Winn tests will be completed on this panel shortly. The special experiments (studies to detect immunity inhibiting processes or reveal subliminal tumor immunity) are substantially larger than the biological characterizations and therefore the panel will be pared to five, a single tumor of each type studied thus far.

Significance to Cancer Research: Apparently, the immunity that can be detected in vitro is ineffectual for tumor rejection in vivo. This project is to examine the role of several processes which might be interfering with tumor rejection immunity. Such information would guide subsequent efforts to improve methods for immunotherapy of cancer.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $81,649

CONTRACT RESEARCH SUMMARY

Title: Immunoprevention of Tumors in Rabbits

Principal Investigator:	Dr. John W. Kreider
Performing Organization:	Pennsylvania State University
City and State:	Hershey, PA
Contract Number:	NO1-CB-74158
Starting Date:	9/29/77
Expiration Date:	9/28/79

Goal: To develop immunologic maneuvers for interfering with the progression of solid tumors from the premalignant to the malignant stage.

Approach: Using the Shope papilloma in the rabbit, a naturally occurring model of neoplastic progression, attempts will be made to intervene in the progression from the preneoplastic stage using nonspecific (BCG, C. parvum) and specific (tumor cell vaccines) immune stimulants.

Progress: Attempts are being made to inhibit transformation of virus-induced papillomas to malignancy. Some animals are being treated by active immunization with papilloma cells and others by injection of a variety of agents into the papillomas. Both approaches have yielded positive effects. It remains to be seen whether or not regression seen so far will be permanent.

Project Officers: Dr. Herbert Rapp and Ms. Judith Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $110,822

CONTRACT RESEARCH SUMMARY

Title: Collection of Sera from Populations with High Cancer Risk

Principal Investigator: Dr. Henry Altschuler
Performing Organization: Philadelphia Geriatric Center
City and State: Philadelphia, PA

Contract Number: NO1-CB-33914
Starting Date: 6/25/73 Expiration Date: 10/24/78

Goal: To collect serial serum specimens from populations at high risk of contracting malignant disease for the purpose of developing and evaluating immunodiagnostic screening tests for human cancer.

Approach: Demographically stable populations at high risk of developing human malignancies in the colon, lung, breast, prostate, bladder, pancreas, etc. will be followed on an annual basis. This includes clinical evaluation and serum collection. The sera will be banked and records kept on the patients so that information obtained from the stored sera can be correlated with development of any particular cancer.

Progress: Over the life of the contract more than 3,000 specimens have been collected and sent to the Mayo Serum Bank for storage. An alphabetical card file is maintained on all subjects as well as separate death index files and cancer index files.

Significance to Cancer Research: Early detection of cancer by means of appropriate testing would permit treatment of cancer at the most favorable time.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $62,789

CONTRACT RESEARCH SUMMARY

Title: Cells Involved in Immune Response to Tumors

Principal Investigator: Dr. W. Hallowell Churchill
Performing Organization: Robert B. Brigham Hospital
City and State: Boston, MA

Contract Number: NO1-CB-64036
Starting Date: 6/30/76 Expiration Date: 6/29/79

Goal: To determine if sensitized lymphocytes stimulated with tumor antigen generate macrophage-activating factor (MAF) or factors capable of enhancing macrophage-mediated cytotoxicity. To define further the nature of the interaction between MAF and macrophages. To explore the mechanisms by which macrophages, once activated, kill tumor cells.

Approach: Macrophages are activated in vitro by treatment in suspension culture with MAF-rich supernatants. Their cytotoxicity for tumor cells labeled with ^{3}H-proline or ^{3}H-thymidine is then determined.

Progress: We have shown that partially purified peritoneal lymphocytes from tumor-immune animals produce macrophage-activating factor (MAF) when stimulated by the immunizing tumor. Further investigation of the role of sugars in the interaction between MAF and macrophages has shown that fucose consistently inhibits the effect of MAF on macrophage cytotoxicity, whereas other sugars such as rhamnose and alpha-methylmannoside do not. Furthermore, removal of cell surface fucose by treating macrophages with fucosidase also inhibits the induction of cytotoxic macrophages by MAF. Cell surface sialic acid may also participate in the MAF macrophage interaction, since treatment of macrophages with neuraminidase renders them refractory to the effects of MAF. Finally, major progress has been made in analyzing the mechanisms by which macrophages, once activated, kill tumor cells. Experiments with inhibitors of protein synthesis show that inhibition of cytotoxic effect roughly parallels inhibition of protein synthesis. Furthermore, binding of tumor cells to activated macrophages is also decreased in the dose-dependent fashion by these inhibitors. Results with inhibitors of microtubules and of microfilaments are similar.

Significance to Cancer Research: Macrophages are an important component of the host response to tumor antigens. Our studies are designed to elucidate the mechanisms by which macrophages acquire enhanced cytotoxicity for tumor cells and the mechanisms by which activated macrophages kill tumor cells.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Facility for Supplying Immune-related Cell Lines

Principal Investigator: Dr. Melvin Cohn
Performing Organization: The Salk Institute
City and State: San Diego, CA

Contract Number: NO1-CB-23886
Starting Date: 2/26/74 Expiration Date: 2/25/79

Goal: To supply the scientific community with immune-related lines important in the study of tumor immunology and to increase the library of useful lines as they are characterized in our laboratory.

Approach: Since this is essentially a contract to supply cell lines, little experimental work is involved. However, the general thrust of the laboratory involved the isolation of immune-related lines of the thymus and bone marrow-derived lineages by the use of leukemia viruses, cell fusions, and chemical carcinogens. As new lines become available, they are introduced into the contract.

Progress: The contractor has shipped over 350 tissue culture and tumor lines to investigators in the scientific community. Over 144 different investigators have received varying numbers of lines in the current contract year. A new catalog (4th edition) of cell lines has been drawn up, reflecting the elimination of all tumor lines (henceforth handled by Litton Bionetics at the request of the Program) and adding a number of new lines which have been demanded greater than ten times. The catalog contains detailed characterization of the cell lines. This contract continues to be an excellent research resource, increasingly utilized by scientists all over the world.

Significance to Cancer Research: The lines in this contract are used by hundreds of laboratories for studies on tumor-specific antigens, antibody structure, immune-related cell functions, somatic genetics, and cell fusions. All of these subjects are major categories under the NCI Cancer Research Program, and the cell lines from this laboratory are key tools in these investigations.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $84,003

CONTRACT RESEARCH SUMMARY

Title: Detection of Tumor-specific Antigens in the Circulation

Principal Investigator: Dr. Gerald N. Callahan
Performing Organization: Scripps Clinic and Research Foundation
City and State: La Jolla, CA

Contract Number: NO1-CB-74126
Starting Date: 9/16/77 Expiration Date: 9/15/79

Goal: To correlate the appearance and quantity of circulating tumor-associated antigens with the stage of development of the tumor and tumor site.

Approach: To detect and quantitate tumor-associated or tumor-specific antigens (TAA) present in sera of mice bearing transplantable carcinomas or sarcomas. To correlate the appearance and quantity of circulating TAA with the stage of development of the tumor. Produce highly specific antisera directed toward TAA to aid in detection of these antigens.

Progress: We have obtained a total of 12 transplantable murine sarcomas and carcinomas induced with physical and chemical agents. These tumors have been expanded, and large numbers of cells from early transplant generations and tissue culture passages have been banked in liquid N_2 for future use. Four of the tumors have been established in continuous culture. Twelve rabbits are currently being injected with both untreated tumor cells and subcellular fractions of tumor cells. These rabbits have begun to produce cytotoxic antibodies, and the specificity of these antisera is being investigated. Also syngeneic mice are being injected with irradiated or iodoacetamide-treated cells. Significant prolongation of survival following tumor challenge (15-30 days over MST) has been observed, and these sera are being evaluated for antibodies reactive with tumor cells.

Significance to Cancer Research: The answers gathered in this system should allow for at least a first step in the assessment of the feasibility of this approach to immunodiagnosis of human neoplasia.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Role of Circulating Tumor Antigens

Principal Investigator: Dr. Joseph D. Feldman
Performing Organization: Scripps Clinic and Research Foundation
City and State: La Jolla, CA

Contract Number: NO1-CB-43874
Starting Date: 3/1/75 Expiration Date: 2/28/80

Goal: To evaluate the effects of immunologic agents and reagents such as antigens, antigen-antibody complexes, and effector cells on the treatment of a virus-induced rat tumor.

Approach: Attempts will be made to augment and exaggerate the host immunologic responses that inhibit or destroy tumor in vivo and to suppress those responses that enhance tumor growth. This entails preparation of (a) membrane antigens of tumor cells; (b) cytotoxic antibodies of a single immunoglobulin class and specificity; and (c) purified populations of cytotoxic effector cells.

Progress: (1) Three species of membrane-tumor-associated antigens (TAA) and four species of cytoplasmic TAA were extracted from the rat Moloney sarcoma tumor.

(2) Immune complexes (IC) of labeled ^{125}I-p30-anti-p30Ab were infused via the heart into MST-bearing rats and controls. IC were eliminated more rapidly in tumor-bearing rats than in controls. Progression, regression, or elimination of MST did not influence metabolism of IC.

(3) Spleen cells with specificity for whole MST were infused i.v. into tumor-bearing irradiated rats. Established tumors were completely eliminated 20-25 days after infusion of spleen cells; tumors grew progressively in rats infused with non-immune spleen cells or in controls given no spleen cells.

Significance to Cancer Research: It is essential to understand the complex interactions of the components of the immune system and to determine how best to manipulate these interactions to achieve a therapeutic benefit in cancer treatment.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $128,726

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy of Mouse Tumors Using Hapten-reactive T-lymphocytes

Principal Investigator: Dr. David Katz
Performing Organization: Scripps Clinic and Research Foundation
City and State: La Jolla, CA

Contract Number: NO1-CB-74196
Starting Date: 9/16/77 Expiration Date: 9/15/79

Goal: To develop an effective method for improving host anti-tumor immunity against growth of syngeneic tumors using hapten-reactive amplifier T cells.

Approach: Mice will be (1) pretreated with hapten-D-GL conjugates and then (2) primed with the specific hapten conjugated to mouse gamma globulin (MGG), and then (3) challenged with hapten-conjugated tumor cells. In therapeutic as opposed to prophylactic models, the solid tumor will be obtained by surgical extirpation or, alternatively, haptenic coupling will be attempted in situ.

Progress: (1) Mice primed in vivo with a TNP-mouse gamma globulin conjugate had in their spleens and lymph nodes TNP-reactive lymphocytes that could be induced to proliferate in vitro upon stimulation with various TNP-conjugates. (2) Spleen cells of mice primed in vivo with TNP-MGG and subsequently inoculated with mitomycin C-treated TNP-modified Thy-56 lymphoma cells generated higher tumor-specific CTL responses upon in vitro restimulation with the same tumor cells than did cells from mice not primed with TNP-MGG. (3) Addition of small numbers of intact or irradiated TNP-MGG-primed spleen cells to cultures of tumor-primed spleen cells augmented the sensitization of the latter aginst TNP-derivatized syngeneic tumor cells as indicated by a marked increase in the tumor-specific CTL response. (4) Intravenous injection of TNP-MGG primed spleen cells mixed with mitomycin C-treated, TNP-derivatized tumor cells had a therapeutic effect on an established lymphoma of mice.

Significance to Cancer Research: The use of hapten-reactive helper T cells may provide a way to augment the immune response to syngeneic tumors that are in general weakly immunogenic, and therefore may enable us to devise better methods for the immunotherapy of cancer.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Purification of Human Tumor-associated Antigens

Principal Investigator: Dr. Ralph A. Reisfeld
Performing Organization: Scripps Clinic and Research Foundation
City and State: La Jolla, CA

Contract Number: NO1-CB-53899
Starting Date: 6/1/75 Expiration Date: 5/31/79

Goal: Development of new methods for the isolation, purification, and physicochemical characterization of human tumor-associated antigens and to make them available for cell-mediated immunity assays and diagnostic testing.

Approach: Human melanoma-associated antigens (MAA) are isolated by mild, least denaturing procedures from cultured melanoma cells and their spent culture media. This includes removal of HLA antigens and lipids and purification and physicochemical characterization of MAA which are defined biologically by their ability to evoke specific cell-mediated and humoral immunity reactions.

Progress: Effective purification procedures developed include fractional salting out, ultracentrifugal flotation in potassium bromide, cation exchange chromatography, affinity chromatography on lectin columns (lens culinaris and ricin), and preparative isoelectric focusing. Purified antigens elicited delayed cutaneous hypersensitivity responses in melanoma patients as well as leukocyte adherence inhibition. Humoral immune responses were documented by inhibition of antibody binding to cultured melanoma target cells as determined in a newly developed radioimmunometric assay. Xenoantisera to purified MAA were produced in rabbits and rendered functionally specific by selective adsorptions with human red cells and cultured human lymphoid cells. These antisera were evaluated by a newly developed radioimmunometric antibody-binding assay. This assay, when used as a binding inhibition test, effectively assessed the serologic activity of MAA, and was used to monitor purification. Purified MAA isolated from spent culture media has an isoelectric point of pH 6.4 and is a glycoprotein with a molecular weight of 58,000, which is not associated with β_2-microglobulin.

Significance to Cancer Research: Availability of purified MAA should prove advantageous to establish their utility to produce specific xenoantibody and to test the efficacy of these reagents for tumor diagnosis, therapy, and in monitoring response to therapy.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy of Mouse Ovarian Cancer Using Specific Serotherapy in Combination with Intraperitoneal C. parvum

Principal Investigator: Dr. Robert C. Bast
Performing Organization: Sidney Farber Cancer Center
City and State: Boston, MA

Contract Number: NO1-CB-74194
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: To evaluate the therapeutic efficacy of specific xenogeneic serotherapy in combination with intraperitoneal C. parvum in the treatment of syngeneic murine ovarian carcinoma transplants.

Approach: Efforts will be made to determine the optimal dose, route, order, and schedule of administration for the treatment combination in mice. Attempts will be made to determine relevant in vitro correlates with disease status. Techniques will be perfected for cryopreservation of human ovarian tumor cells and for the preparation of specific xenoantiserum against human tumor cells for possible use in human clinical trials at some future date.

Progress: A combination of C. parvum and a specific xenoantiserum has been found significantly more effective than either single agent in prolonging survival of mice that had received syngeneic ovarian carcinoma cells intraperitoneally. In vitro, a combination of C. parvum activated peritoneal cells and specific xenoantiserum has also proved significantly more effective than either single component in destroying murine ovarian carcinoma cells in the absence of complement.

Techniques have been developed for the separation and cryopreservation of human ovarian cells. Twenty B-lymphocyte cell lines and 4 epithelial ovarian tumor cell lines have been established. A xenoantiserum raised against one of the tumor lines and absorbed exhaustively with an autochthonous B-lymphocyte line binds to the patient's tumor cell line and to cryopreserved tumor cells or lines from at least 3 other ovarian cancer patients.

Significance to Cancer Research: Synergy between two different forms of immunotherapy may permit more effective treatment of human cancer.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $126,143

CANCER RESEARCH SUMMARY

Title: Hybridization Techniques To Obtain Functional T Cells

Principal Investigator: Dr. Harvey Cantor
Performing Organization: Sidney Farber Cancer Institute
City and State: Boston, MA

Contract Number: NO1-CB-74147
Starting Date: 9/16/77 Expiration Date: 9/17/80

Goal: Produce continuously propagatable lines of antigen-specific T-cell subclasses, each expressing a distinct immune function.

Approach: Separation of T cells into functionally distinct sets; generate highly purified antigen-reactive T cells from these subsets; induce propagatable, cloned cell lines following hybridization with appropriate T-cell tumors.

Progress: This contract is completing its second year. Progress has been excellent in several areas. Two methods have been developed that allow specific positive selection of T cells expressing different Ly and Qal components. Another technique has been developed which makes it possible to derive a clone of anti-SRBC specific T_H cells. Experimentally, two of nine such clones retained activity and clonality was verified by Y-chromosome analysis. A major difficulty encountered in growth of T-cell clones is the inability of such cells to grow in culture in numbers greater than 10^5 cells/well, and, in order to increase that number of cells, the contractor has methodically analyzed the requirements for continued cell growth. The group has shown that increased cell saturation densities can be achieved (up to 10-fold) if increased concentrations of nonessential amino acids like arginine and asparagine, as well as folic acid, are added to the media. Ly 23 cells were obtained from "HIS" donors and put into culture under conditions allowing clonal growth. Although the culture contains Thy 1^+ Ly 23^+ cells, it has not yet been determined if this is a single clone, although dilution used for seeing these cultures suggests that this is the case.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Isolation and Tissue Culture of Human Tumor Cells

Principal Investigator: Dr. Jorgen E. Fogh
Performing Organization: Sloan-Kettering Institute
City and State: New York, NY

Contract Number: NO1-CB-43854
Starting Date: 8/8/73 Expiration Date: 8/7/79

Goal: To develop further procedures for encouraging tumor cell growth in culture and to perform tests necessary to define standard cell lines from various tumors.

Approach: Human tumor cell lines are established in culture from surgical specimens and are collected from other investigators. They are characterized in this laboratory as to malignancy, cell type, and special features as needed for individual investigations.

Progress: This contract supports a resource in the form of an extensive bank of human tumor lines grown in vitro. Cell lines are preserved after as few passages as possible and are characterized in terms of morphology, nutritional requirements, growth in vitro, chromosomal characteristics, poliovirus susceptibility, and isozyme patterns. Such characterization enables the investigators to determine possible cross contamination with other cell lines, microbial contamination, and stability. The 418 cultured human tumor cell lines are available to other investigators. Five new lines have been added during the past 11 months with efforts to add normal cell lines from the same patients who are sources of tumor material. The bank now includes 17 carcinomas of the lung, 31 of the breast, 18 of the colon, 20 lines of brain and nervous tissue origin, 10 of the kidney, 19 of the bladder, 2 of the prostate, and 69 melanomas. Fifty-seven of the 392 lines are of non-tumor origin. Two hundred ninety-nine shipments have been made to investigators in an 8-month period, indicating a good utilization of the resource.

Significance to Cancer Research: The collection of well-characterized contamination-free human tumor cell lines is pertinent for many disciplines of experimental and clinical cancer research.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $125,941

CONTRACT RESEARCH SUMMARY

Title: Human Tumor-associated Antigens and Corresponding Antibodies

Principal Investigator: Dr. Yashar Hirshaut
Performing Organization: Sloan-Kettering Institute
City and State: New York, NY

Contract Number: NO1-CB-53942
Starting Date: 6/30/75 Expiration Date: 12/31/78

Goal: To demonstrate new tumor-related antigens associated with human sarcomas. Antigens found, as well as those already demonstrated, are to be characterized to determine their nature, specificity, and distribution.

Approach: The serologic responses of patients with sarcoma to their tumor cells are assayed by immunofluorescence, complement fixation, and microcytotoxicity tests. Preliminary characterization of antigens is achieved by immunologic means. Efforts are under way to isolate, purify, and biochemically analyze specific antigens.

Progress: The conditions for growing human sarcomas in culture and maintaining antigen production have been established. Of particular interest has been the requirement for human cord serum as the source of complement in the S_3 assay. Guinea pig complement is usually inactive or gives very low titer reactions. Collaborative studies have revealed that there are quantitative differences in the components present in the complements of different species, but consumption studies so far show no differences in their attachment to the sarcoma antigen-antibody complexes. S_1 and S_3 are now clearly established as heterophile antigens unrelated to those associated with sheep red blood cells such as the Forssman, infectious mononucleosis, and serum sickness antigens. Antibody to serum sickness antigen has, however, also been found to be more prevalent in patients with malignancy. Leukemic blasts have heterophile antigens present on their surface which are not found on more mature cells. Such antigens are also associated with oncornavirus isolates. Efforts at biochemical purification of S_3 are under way and indicate that it may be structurally distinct from the cross-reacting antigen present in guinea pig kidney cells.

Significance to Cancer Research: Definition and characterization of human tumor antigens should be of value in permitting early diagnosis and the further development of immunotherapy for cancer. Such antigens may also provide new insight into changes that accompany malignant transformation.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Phase I Study of Effects of Immune Stimulants on Human Immune Response

Principal Investigator: Dr. Yashar Hirshaut
Performing Organization: Sloan-Kettering Institute
City and State: New York, NY

Contract Number: NO1-CB-53970
Starting Date: 5/16/75 Expiration Date: 5/15/80

Goal: To evaluate selected immunotherapeutic agents for acute and chronic toxicity, maximum tolerated dose, and effects on immune status. The agents presently being investigated are Corynebacterium parvum (C. parvum), levamisole, BCG, intravenously administered, and purified endotoxin.

Approach: Clinical protocols have been designed that provide for the administration of new immunotherapeutic agents to patients in accordance with acute, chronic, and escalating dose schedules. Where feasible, the routes of administration include oral, parenteral, and intralesional. During treatment, toxic and immune parameters are measured at regular intervals.

Progress: During the last year the contractor has exhaustively analyzed toxicity and immunology data on the computed C. parvum and levamisole trials. The most common side effects of C. parvum were fever, chills, nausea, and vomiting. Immunologically, the C. parvum-treated patients show a significant increase in IgM, absolute lymphocyte count, and the number of granulocytes reducing NBT. There was also a decrease in response to Con A.

Toxicities to levamisole include nausea, lethargy, cramps, and diarrhea. Immunologically, the levamisole-treated patients showed an increased lymphocyte response to PHA and a decreased response to Con A. Clq tended to decrease, whereas in response to increasing dose of levamisole, uric acid levels rose.

Seven patients have been entered on the intravenous BCG trial, and patient entry is expected to begin soon on the endotoxin and Poly ICLC trials.

Significance to Cancer Research: Immunotherapy is a new modality of treatment for cancer whose appropriate use depends on understanding of how available agents affect specific aspects of immune function. Phase I studies underway should assist in determining optimal doses producing acceptable toxicity.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $95,895

CONTRACT RESEARCH SUMMARY

Title: Plasma Therapy of Mouse Tumors

Principal Investigator: Dr. Robert L. Kassel
Performing Organization: Sloan-Kettering Institute
City and State: New York, NY

Contract Number: NO1-CB-74197
Starting Date: 9/30/77 Expiration Date: 9/29/79

Goal: To determine whether plasma therapy (alone or in combination with other treatment modalities, e.g., chemotherapy) will be useful in the treatment of transplantable solid tumors and in the prevention and treatment of spontaneous leukemia in mice.

Approach: Leukemic or preleukemic mice will be treated with $C5^+$ heparinized plasma or purified eluates and their survival compared with control animals treated with $C5^-$ heparinized plasma or purified $C5^-$ eluates. Plasma therapy will also be combined with chemotherapy and/or other treatment modalities. Circulating immune complexes will be assayed before, during, and after therapy.

Progress: Plasma therapy in preleukemic mice appears to result in a slight delay in time of leukemia development in animals treated with CD-1 ($C5^+$) eluates and a slight acceleration in animals treated with AKR ($C5^-$) eluates when compared with saline-treated controls. Combined chemo-plasma therapy results in 25% cures and a reduction in chemotherapy dosage of almost 70%. Eluates from the heparin plasma precipitate (at 4°C) have been found to contain CIg (cold insoluble globulin or fibronectin) and purified CIg appears to have similar antileukemic activity.

Significance to Cancer Research: In spite of toxic side effects, chemotherapy has been a treatment of choice because it can prolong life and in some cases "cure." Any adjunct to chemotherapy that reduces the total dose required for effective cytoreduction should reduce the toxic burden the host must bear. The synergistic action of cytoxan-plasma therapy may present a method of reducing burden and prolonging remission.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: In Vitro Augmentation of Cell-mediated Cytotoxicity

Principal Investigator: Dr. Philip Livingston
Performing Organization: Sloan-Kettering Institute
City and State: New York, NY

Contract Number: NO1-CB-74130
Starting Date: 9/30/77 Expiration Date: 9/29/79

Goal: To develop means to augment cell-mediated cytotoxicity.

Approach: Exposure of melanoma patient lymphocytes to autologous melanoma cells in vitro under various experimental conditions; determination of the specificity of antigens detected in cytotoxicity tests by cellular immunoadsorption and inhibition tests with fixed and unfixed tissue; and characterization of melanoma-specific effector cells as opposed to natural killer cells and suppressor cells.

Progress: The goal of this contract is to augment cell-mediated cytotoxicity (CMC) against autologous human melanoma by in vitro sensitization. The characteristics of the effector cell and the specificity of the antigens involved are to be determined. Eighteen additional tumor lines from human malignant melanoma have been established in the last year. Co-culture of patient's lymphocytes with autologous melanoma cells generally reduced rather than augmented cell-mediated cytotoxicity. Co-culture with allogeneic B cells increased CMC for autologous and allogeneic melanoma cells. Effector cells in most instances are T cells although isolated instances of non-T effectors are seen.

Significance to Cancer Research: We expect this study to contribute to our understanding of cellular immunological rejection mechanisms, and to the development of methods for strengthening these mechanisms.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $100,683

CONTRACT RESEARCH SUMMARY

Title: Diagnostic Application of Human Tumor-associated Antigens

Principal Investigator: Dr. Kenneth O. Lloyd
Performing Organization: Sloan-Kettering Institute
City and State: New York, NY

Contract Number: NO1-CB-64021
Starting Date: 6/30/76 Expiration Date: 6/29/79

Goal: The aim of this project is to purify human ovarian cancer antigens which can be detected using heterologous antisera and to develop sensitive assays for these antigens. These assay systems will then be used to detect the antigens in sera of women with ovarian cancers and of suitable controls to determine their usefulness in the diagnosis of ovarian cancer.

Approach: The approach entails the use of heteroimmune sera to detect and assay tumor-associated antigens in ovarian tumors and sera from cancer patients.

Progress: Investigations on two antigens (OVC-1 and OVC-2) defined by heterologous sera prepared against extracts of human ovarian tumor extracts have continued. OVC-1, which was found in 48 of 93 ovarian cancers, has been shown to be a new pregnancy-associated antigen. Partial purification of this antigen has been achieved; it exists in two forms having molecular weights of about 200,000 and 30,000, respectively; the larger form has a reduced subunit size of 60-80,000 daltons. OVC-2, which was found in 8 of 93 ovarian cancers, has been identified as a new fetal antigen. It has been purified to a high degree of homogeneity and is a glycoprotein with a molecular weight of about 45,000 daltons. Purified OVC-2 is being used to prepare high titered, specific antisera suitable for use in radioimmunoassays. Both OVC-1 and OVC-2 have recently been found in amniotic fluid and this may well serve as a convenient source of antigen in the future.

Significance to Cancer Research: Ovarian cancer has a high death rate because diagnosis and surgical removal are often critically delayed due to its deep location and lack of early symptoms. Immunodiagnostic assays are aimed at the early detection of ovarian tumors in asymptomatic women.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Serologic Assays with Purified Melanoma-associated Antigens

Principal Investigator: Dr. Kenneth O. Lloyd
Performing Organization: Sloan-Kettering Institute
City and State: New York, NY

Contract Number: NO1-CB-74127
Starting Date: 9/30/77 Expiration Date: 9/29/79

Goal: Develop an assay for new tumor-associated antigens.

Approach: Isolate and characterize cell surface antigens of human malignant melanoma and develop a sensitive serologic assay for monitoring and diagnosis of melanoma.

Progress: This contract is completing its second year of work isolating and characterizing cell surface antigens of human malignant melanoma with the ultimate intent to develop a serologic assay for diagnosis and monitoring of the disease. Heterologous anti-melanoma sera have been prepared against antigens originally defined using serological assays in autologous melanoma systems. By these assays it has been shown that human melanoma possesses melanoma-specific antigens. Antisera to three different antigens have been prepared. One antiserum is against whole melanoma cells, which recognizes a common antigen shared between melanoma and other tumor cells. A second antiserum is prepared against a "shed" glycoprotein sample known to contain a melanoma antigen detected by the autologous typing approach. The third antiserum is prepared against a cell surface glycoprotein that binds both Con A and WGA lectins.

Significance to Cancer Research: New sensitive assays are needed for the immunodiagnosis and monitoring of malignant melanomas.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $64,114

CONTRACT RESEARCH SUMMARY

Title: Evaluation of Immunotherapy with Tumor Preparation in Man

Principal Investigator: Dr. Herbert F. Oettgen
Performing Organization: Sloan-Kettering Institute
City and State: New York, NY

Contract Number: NO1-CB-64062
Starting Date: 6/16/76 Expiration Date: 6/15/80

Goal: To determine the effectiveness of treating patients with advanced malignant melanoma with a combination of surgery, BCG, autologous tumor cell vaccine, and chemotherapy (DTIC). To monitor the patients' immune response to surface antigens of autologous and allogeneic melanoma cells, as measured by serological and cellular reactions, and to correlate the response with the course of the disease and the effects of therapy.

Approach: Patients with malignant melanoma Stage III will be treated with BCG and a vaccine of autologous irradiated tumor cells. Patients with malignant melanoma Stage IV will receive BCG, irradiated autologous tumor cells, and DTIC. The patients' immune response to surface antigens of their own tumor cells will be tested in vitro by serological tests (mixed hemadsorption, immune adherence, complement-dependent cytotoxicity, antibody-directed cell-mediated cytotoxicity), and tests for lymphocyte cytotoxicity on autologous or allogeneic target cells.

Progress: During the past year 28 new continuous melanoma cell lines were established. Five patients received autologous melanoma cell vaccine, bringing the total to 18. In the serological study of these patients an increase in titer of antibodies to autologous melanoma cell surface antigen was seen in 8 patients following vaccine administration. Extensive absorption analysis showed the specificity of these antibodies was restricted to melanoma cell surface antigens in 2 patients, and in 6 patients extended to other types of culture of cells. In 4 cases of the latter group, the antibodies were directed against components of fetal calf serum. The clinical course of disease of the 2 patients with melanoma-specific autologous antibodies was reported as unexpectedly favorable.

Serological study of 17 patients receiving allogeneic melanoma vaccine is under way.

Significance to Cancer Research: Malignant melanoma offers unique opportunities for study in that the serology is further advanced than in other types of cancer. Thus a trial of autologous vaccine will provide information concerning therapy as well as basic human cancer immunology.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $140,633

CONTRACT RESEARCH SUMMARY

Title: Immunization with BCG and Allogeneic Renal Cancer Cells in Patients with Renal Cell Cancer

Principal Investigator: Dr. Herbert F. Oettgen
Performing Organization: Sloan-Kettering Institute
City and State: New York, NY

Contract Number: NO1-CB-74145
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: To determine whether the cells of renal cancers have cross-reacting tumor-associated antigens.

Approach: Assays for humoral immunity to autologous tumor cells will be performed before and after immunization with BCG and allogeneic renal cancer cells. Attempts will be made to analyze the specificity of the serological reactions and to investigate the patterns of antigen expression in terms of culture time, growth characteristics, and cell cycle kinetics. Periodic assessment of humoral immunity will be performed on sera from patients with Stage I renal cancer receiving conventional therapy and on sera from patients with Stage II, III, and IV renal cancer who are immunized with BCG and allogeneic renal cancer cells.

Progress: Thirteen patients have been treated with allogeneic renal cancer cell vaccine and BCG. The choice of cell lines for the preparation of vaccines was based on a satisfactory growth rate and on the expression of shared renal cancer cell surface antigens.

To provide target cells for these studies renal cancer tissue from 80 patients and normal kidney tissue from 47 patients have been placed in culture.

In a serological survey, antibody to autologous renal cancer cells was detected in 23 of 28 patients by the immune adherence assay, in 1 of 22 patients by the mixed hemadsorption assay, and in 14 of 15 patients by the protein A assay. In extensive absorption analysis of sera from 6 patients, 7 surface antigenic systems defined by autologous typing have been characterized. The frequency of antibody to shared (class II) antigens was higher than in the investigator's corresponding studies of melanoma, leukemia, and astrocytoma.

Significance to Cancer Research: The interest in clinical immunotherapy has emphasized the need for specific and reproducible methods for monitoring the cancer patient's immunological reactivity to cancer cells. In addition, serology appears perhaps the most direct approach to determining the form and source of tumor antigens to be used in clinical trials of active immunotherapy for cancer.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $124,382

CONTRACT RESEARCH SUMMARY

Title: Therapy of Tumors in Mice with Tumor Necrosis Factor (TNF)

Principal Investigator: Dr. Lloyd Old
Performing Organization: Sloan-Kettering Institute
City and State: New York, NY

Contract Number: NO1-CB-64051
Starting Date: 6/30/76 Expiration Date: 6/29/79

Goal: To determine the effectiveness of tumor necrosis factor (TNF) in the therapy of transplantable and spontaneous mouse tumors.

Approach: TNF is found in the serum of BCG or C. parvum primed mice injected with endotoxin. Both TNF^{+} serum and partially purified TNF are being tested for antitumor activity in the following five mouse tumor systems: Meth A sarcoma (BALB/c); leukemia L1210 (DBA/2); sarcoma 19B-MCA (C57BL/6); AKR spontaneous leukemia; and C3H spontaneous mammary carcinoma. Tumor necrosis, tumor growth, inhibition of metastases, and survival are the parameters to be assessed.

Progress: Standard methods have been devised for the production and partial purification of tumor necrosis factor. Studies have been conducted on the antitumor activity of TNF^{+} serum in several mouse tumor systems. The investigators are evaluating the effects of single vs. multiple injections of TNF^{+} serum and concentrated, purified G-200 fractions of TNF^{+} serum. Among the tumor systems studied there appears to be some variability in the effects of treatment with TNF^{+} serum. For instance, growth of leukemia L1210 was not affected by treatment with TNF, while Meth A and 193-MCA solid tumors were necrosed after single injections of TNF^{+} serum.

Significance to Cancer Research: Agents such as BCG and C. parvum, which augment immunological reactivity in a general fashion under certain circumstances and have tumor inhibitory activity in experimental systems, are receiving greatest attention in clinical trials of cancer immunotherapy. The past work leads to speculation that TNF may be one of the mediators responsible for the antitumor activity of these microbial immunopotentiators. Understanding how TNF causes tumor cell destruction and determining how effective it is as an antitumor agent may provide novel approaches to cancer immunotherapy.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Evaluation of C. parvum in Disseminated Carcinoma of the Breast

Principal Investigator: Dr. Carl M. Pinsky
Performing Organization: Sloan-Kettering Institute
City and State: New York, NY

Contract Number: NO1-CB-53873
Starting Date: 6/30/75 Expiration Date: 6/29/80

Goal: To determine whether the addition of Corynebacterium parvum to combination chemotherapy in patients with advanced breast cancer increases response rate, increases response duration or survival time, protects against the deleterious effects of chemotherapy, or augments immunologic function.

Approach: This is a prospective, randomized trial using cyclophosphamide, adriamycin, methotrexate, and 5-fluorouracil every 28 days, with or without C. parvum given weekly, subcutaneously.

Progress: One hundred five patients have been entered on this randomized study; 59 patients received chemotherapy only; 61 received chemotherapy plus C. parvum. Of 53 evaluable patients receiving chemotherapy, only 28 have had a complete or partial response. Of 54 evaluable patients receiving chemo-immunotherapy, 20 have had complete or partial response. There is at present no significant difference in response rate or response duration or survival between the two groups. Leukopenia and thrombocytopenia have been similar in the two groups. Immunological activity in the two groups has been similar.

Significance to Cancer Research: Since complete response rate is minimal and ultimate survival continues to be short in patients with advanced breast cancer, all leads which suggest lengthened survival must be followed.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $66,247

CONTRACT RESEARCH SUMMARY

Title: Intratumoral BCG Prior to Radiation and Cystectomy in Patients with Bladder Cancer

Principal Investigator: Dr. Carl M. Pinsky
Performing Organization: Sloan-Kettering Institute
City and State: New York, NY

Contract Number: NO1-CB-74142
Starting Date: 9/16/77 Expiration Date: 9/15/79

Goal: To evaluate the therapeutic efficacy of intratumoral injection of BCG prior to radiation and cystectomy.

Approach: Eligible patients with solitary infiltrating bladder cancers will be randomly allocated to treatment with intratumoral injection of BCG prior to radiation and cystectomy, or to treatment with radiation and cystectomy alone. The study will evaluate the effects of BCG therapy on disease-free interval, site(s) of recurrence, and survival.

Progress: (Pinsky--contract 74142) Patient accrual has been lower than expected in that after 16 months, 19 patients have been entered into the study: 9 received BCG and 10 were control patients. It is too soon to analyze antitumor effects. Necrosis of tumor and foreign body granuloma have been seen in the pathologic specimens of BCG-treated patients, but it has not been possible to attribute this to the therapy. Toxicity following the injection of BCG has consisted of chills and fever with some sweating in 8/9 patients. Transient abnormalities of hepatic enzymes have also been seen. One BCG-treated patient with a history of prior nonfatal myocardial infarctions died of a myocardial infarction five weeks after the BCG injection and three weeks after the radical cystectomy.

Significance to Cancer Research: More effective therapy for bladder cancer is needed. It is important to determine whether an approach that is successful in animal models will be useful in the therapy of humans.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: BCG Immunotherapy in Patients with Recurrent Superficial Bladder Cancer

Principal Investigator: Dr. Carl M. Pinsky
Performing Organization: Sloan-Kettering Institute
City and State: New York, NY

Contract Number: NO1-CB-74146
Starting Date: 9/16/77 Expiration Date: 9/15/80

Goal: To evaluate the therapeutic efficacy of intravesical and percutaneous BCG in patients with recurrent superficial bladder cancer.

Approach: A controlled, randomized, prospective study will be undertaken in which the administration of BCG shall be identical to the methods published in Journal of Urology 116:180-183, 1976. The study will assess the effect of BCG therapy on the rate of tumor recurrence.

Progress: A total of 33 patients have been randomized at this time; 17 for fulguration alone and 16 for fulguration followed by BCG. No severe toxicity has been seen in the BCG-treated patients to date, but dysuria, hematuria, frequency, and urgency have been common. It is too early to assess therapeutic effects.

Significance to Cancer Research: A pilot study has suggested that intravesical and percutaneous BCG decreases the number of recurrences in patients with superficial bladder carcinoma. It is important to establish that this is the case with a controlled, prospective, randomized trial.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $31,574

CONTRACT RESEARCH SUMMARY

Title: HLA Typing of Human Tissue Culture Cell Lines

Principal Investigator: Dr. Marilyn Pollack
Performing Organization: Sloan-Kettering Institute
City and State: New York, NY

Contract Number: NO1-CB-84255
Starting Date: 9/30/78 Expiration Date: 9/29/79

Goal: Determine the HLA antigens present on some frequently used human tissue culture cell lines derived from tumors and relate them to the HLA phenotype of lymphocytes from the cell line donor, where possible.

Approach: Perform HLA typing using a modification of the complement-dependent microcytotoxicity procedure. The HLA-A, -B and -C locus antigens expressed on at least 45 frequently used human tissue culture cell lines will be identified and DR locus antigens will be screened for.

Progress: New contract.

Significance to Cancer Research: Identification of HLA antigens on the surface of human tumor tissue culture cells may be useful in the identification and characterization of tumor immunodiagnosis associated antigens on such cells.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Cells Involved in Immune Response to Tumors

Principal Investigator: Dr. Fung-Win Shen
Performing Organization: Sloan-Kettering Institute
City and State: New York, NY

Contract Number: NO1-CB-64031
Starting Date: 6/16/76 Expiration Date: 6/15/79

Goal: To develop a system for measuring the immune capability of T cells against syngeneic chemically induced tumors of C57BL/6 mice in vivo. By use of this system to delineate the contribution of tumor-specific T cells to immunity against tumors in vivo. To determine which functionally distinct T-cell populations may be responsible for abrogating this destruction in vivo. To determine whether any cross-reactions of different chemically induced tumors can be demonstrated by cellular as well as serological methods.

Approach: The in vivo assay will be applied to a series of chemically induced C57BL/6 transplanted tumors to establish the role of the Lyt-1, Lyt-23, and Lyt-123 functional subclasses, alone and in combination, in the immune response to chemically induced tumors in vivo.

Progress: Further evidence that the Lyt-123 set is necessary for rejection, under the conditions of the Winn assay, has come from experiments showing that neither the Lyt-1 set alone nor the Lyt-23 set alone, nor any combination of these two sets, in feasible numbers, will completely suppress Meth 4 sarcoma in syngeneic hosts. We do not infer that Lyt-123 is the effector-killer cell in this system, but rather that Lyt-1 amplifiers and Lyt-23 killer-effectors are drawn from a specifically primed Lyt-123 pool. The grounds for this conclusion are Stutman's work on cytotoxicity against syngeneic mammary tumor cells in vitro, in which the Lyt-123 set is required for maximal cytotoxicity in a long-term assay, despite the fact that the killer-effectors at all phases of cytotoxicity have the phenotype Lyt-23.

Significance to Cancer Research: A foreseeable outcome of this study is that elimination of one functionally distinct Lyt cell population may augment tumor rejection, and another suppress it. At this point it would not be difficult to envisage research programs directed to the application of such methods to therapy of at least some human cancers.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Evaluation of Levamisole as a Therapeutic Adjunct in Squamous Cell Carcinoma of the Head and Neck

Principal Investigator: Dr. Elliot W. Strong
Performing Organization: Sloan-Kettering Institute
City and State: New York, NY

Contract Number: NO1-CB-53875
Starting Date: 6/30/75 Expiration Date: 6/29/80

Goal: To evaluate the usefulness of levamisole in the treatment of patients with squamous cell cancer of the head and neck.

Approach: Patients stratified according to type of disease, primary or recurrent, stage and site, are randomized after surgery on a double-blind basis to receive levamisole or a placebo. Treatment is given three times on alternate weeks for two years. The effects of levamisole on clinical course as well as on immune status are evaluated.

Progress: Of the 97 patients entered in the study, 74 are evaluable. All evaluable patients had squamous cell carcinoma of the oral cavity, pharynx, or larynx and were free of detectable cancer after surgery for recurrent or primary disease. Thirty-five evaluable patients received levamisole and 39 received placebo. Preliminary results indicate no difference in time to recurrence in the two treatment groups. In the group of 13 patients with primary operable disease, Stage II, there is a statistically significant prolongation of time to recurrence for the levamisole group. Due to the small number of patients and relatively short follow-up time, this result must be viewed with caution. There were no significant differences in the results of serial immunological tests between the two groups of patients.

Significance to Cancer Research: Immunopotentiation is a promising clinical approach to cancer therapy especially when used as an adjunct in patients with known immunosuppression. Levamisole, an immune modulator, has been reported to be useful in the treatment of resectable lung cancer and in patients treated for locally advanced breast cancer with radiation (Rojas et al.). This study will attempt to determine whether levamisole has therapeutic efficacy in patients with head and neck cancer.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $82,120

CONTRACT RESEARCH SUMMARY

Title: Direct Assay for Lymphokine

Principal Investigator: Dr. Zoltan Lucas
Performing Organization: Stanford University
City and State: Stanford, CA

Contract Number: NO1-CB-84263
Starting Date: 9/30/78 Expiration Date: 9/29/79

Goal: Development of more rapid and quantitative methods than currently available for assaying lymphokines.

Approach: Prepare reagents and develop sensitive radioimmunoassays for α-LT, β-LT, and IDS from human lymphocytes.

Progress: This project is completing its first contract year. The workscope calls for preparation of reagents for and development of a sensitive radioimmunoassay for lymphotoxins. The group has purified α-LT (α-lymphotoxin) from culture supernatant fluids of a lymphoblast line and tonsil lymphocytes. They are using a simple purification procedure incorporating column chromatography, which is readily adaptable to larger scale preparation of reagents. A total of 8.2 mg of α-LT has been prepared and 560 µg have been ^{125}I-labeled. Tests for homogeneity of the α-LT preparation, manufacture of third-generation rabbit antibody to α-LT, and modification of the double antibody precipitation assay for ^{125}I-α-LT by inhibition of binding of unlabeled α-LT are currently under way.

Progress has been good in the first months of contract work and it is anticipated that assays for β-LT on material from NCI can be performed shortly. A limited amount of labeled β-LT and second generation anti-β-LT is anticipated to be available for NCI-designated recipients by June 1979. Plans include β-LT studies on specimens from cancer patients and preparation of IDS (inhibitors of DNA synthesis) reagents to develop a RIA for IDS. A decision will have to be made by Program Staff regarding disposition of the reagents.

Significance to Cancer Research: Improvement of methods for cancer detection, diagnosis, and screening.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $155,034

CONTRACT RESEARCH SUMMARY

Title: Cytotoxic Activity of Syngeneic Complement

Principal Investigator: Dr. Leon Rosenberg
Performing Organization: Stanford University
City and State: Stanford, CA

Contract Number: NO1-CB-84245
Starting Date: 9/30/78 Expiration Date: 9/29/79

Goal: Study the interactions of tumor cell, anti-tumor cell antibody, and complement in completely autologous or syngeneic systems comparing normal, premalignant, and malignant cells.

Approach: Identify, characterize, and attempt neutralization of a proposed membrane-associated factor responsible for blocking activity of syngeneic complement against tumor target cells.

Progress: New contract.

Project Officers: Dr. Herbert Rapp and Ms. Judith Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $90,213

CONTRACT RESEARCH SUMMARY

Title: Early Detection of Antibodies to Syngeneic Mouse Tumors

Principal Investigator: Dr. Isaac P. Witz
Performing Organization: Tel Aviv University
City and State: Tel Aviv, Israel

Contract Number: NO1-CB-74134
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: Study alterations in the serologic reactivity pattern in mice at high risk of developing carcinogen-induced or endogenous virus-induced malignancies as well as reactivity of such mice toward antigens associated with the relevant tumors.

Approach: Use the following animal model systems: spontaneous tumors in C3HeB mice; mammary tumors induced by DMBA in BALB/c mice; and lung adenomas in urethan-treated BALB/c mice.

Progress: This contract is completing its second year of work, studying alterations of serological reactivity pattern in mice at high risk of developing malignancies. Breeding colonies of the appropriate mice have been set up for these longitudinal studies. The serologic pattern of normal mice has been established to serve as a basis for comparison of altered reactivity. Various technical and methodological aspects have been analyzed to understand what effects bleeding, aging, and unrelated immunizations may have on the anti-tumor serologic reactivity of these mice. These baseline studies will enable the contractor to do the analyses of the altered serologic reactivities as carcinogen- and viral-induced malignancies develop.

Significance to Cancer Research: The results will indicate whether or not altered serologic activity against tumor-associated antigens signals the appearance of primary or transplanted tumors.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $100,300

CONTRACT RESEARCH SUMMARY

Title: In Vitro Induction of Functional Differentiation of T Cells in Tumor Immunology Systems

Principal Investigator: Dr. Samuel D. Waksal
Performing Organization: Tufts University
City and State: Boston, MA

Contract Number: NO1-CB-74150
Starting Date: 9/16/77 Expiration Date: 9/15/80

Goal: Study the role of thymus epithelium in the differentiation and transformation of lymphocytes, including isolation and characterization of prothymocytes from normal, athymic, and tumor-bearing mice.

Approach: Presentation of tumor and virus antigens to prothymocytes during in vitro differentiation to produce populations of antitumor effector cells. Analysis of the ability of thymus epithelium to induce T-lymphocyte differentiation in high leukemic strains (AKR/J and HRS/J) as opposed to control strains.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Diagnostic Applications of Human Tumor-associated Antigens

Principal Investigator: Dr. Ilkka P. T. Hakkinen
Performing Organization: Turku University
City and State: Turku, Finland

Contract Number: NO1-CB-64070
Starting Date: 6/30/76 Expiration Date: 6/29/79

Goal: To evaluate the FS antigen (fetal sulfoglycoprotein antigen) in screening for early gastric cancer. To characterize the FS antigen and standardize the reaction, a trial mass-screening for gastric cancer is being carried out among an unselected population. Clinical material is used to test the FSA method in discriminating between benign and malignant gastric ulceration.

Approach: Acid glycoproteins from gastric juice are tested by immunodiffusion against FS-antiserum, its preparation being standardized as far as possible. Subjects analyzed as secreting FSA are examined for gastric alterations by gastroscopy, and the entire cohort followed for two years. Cancer rates will be compared with actual statistics.

Progress: More than 95% of clinical gastric cancers were earlier found to secrete FSA in gastric juice. On the other hand, less than 10% of the people over 40 secrete FSA. Results of this mass-screening trial on 53,020 subjects seem to support the hypothesis that FSA secretors form a high-risk group for gastric cancer; the majority of cancers were found in the small minority (8.7%) of FSA-positive subjects. Figures for prognosis are not yet available, but there were a great many early cancers among the gastric cancers found in FSA secretors.

Significance to Cancer Research: If the method is proved to be useful, it will provide a new tool for early diagnosis of gastric cancer.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $19,580

CONTRACT RESEARCH SUMMARY

Title: Animal Models for Marrow Transplantation

Principal Investigator: Dr. Paavo Toivanen
Performing Organization: Turku University
City and State: Turku, Finland

Contract Number: NO1-CB-74177
Starting Date: 9/30/77 Expiration Date: 9/29/79

Goal: Study the origin and early differentiation of lymphoid stem cells in chickens.

Approach: Transplantation of stem cells or allogeneically educated, more mature cells into immunodeficient chicks or chicken embryos. Study the fate and function of transplanted cells by morphological and functional means. Determine whether stem cells originate in the yolk sac or intra-embryonically.

Progress: This contract is completing its second year. Evidence has been obtained to indicate that embryonic bone marrow cells do not enter the bursa in significant numbers; still they home and proliferate in the bone marrow and spleen of embryonic recipients and develop into mature B (anti-Ig responsive) as well as T (Con A responsive) lymphocytes. This is supported by experiments in which marked embryonic bone marrow cells were transplanted into embryonic recipients. Apparently in addition to the bursa, the spleen and bone marrow represent alternate pathways for B-cell differentiation. Studies to elucidate this possibility are under way. No evidence for T-cell precursors in the 18-day embryonic bursa has been found in studies using transplantation of chromosomally marked cells. Other studies indicate that bursal stem cells develop to mature post-bursal cells also within an allogeneic bursa which seems to demonstrate that the interaction between lymphoid cells and stromal cells in the bursa is not genetically restricted. Earlier observations that lymphoid stem cells originate primarily in the embryo itself and not in the yolk sac have been confirmed using chicken lines congenic for the IgG allotype by transplantation of two-day embryos on age-matched yolk sacs. Early stem cells seem to derive from the intra-embryonic blood islands.

Significance to Cancer Research: In order to understand disturbances in the immune surveillance system facilitating the development of malignancies, and to be able to manipulate the system therapeutically, detailed knowledge of its normal development is of fundamental importance.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $43,500

CONTRACT RESEARCH SUMMARY

Title: Cell Surface Immunobiology of Metastatic Tumors

Principal Investigator: Dr. Garth L. Nicolson
Performing Organization: University of California
City and State: Irvine, CA

Contract Number: NO1-CB-74153
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: To study surface properties of tumor cells important in malignant tumor spread.

Approach: Use or derive rat and/or mouse variant cell lines with altered in vivo properties such as the ability to metastasize via lymphatics or blood to specific secondary sites.

Progress: The project is in its second year. Using their newly developed lectin immobilization techniques, they have begun isolation of a 70,000 MW compound which is an exposed cell surface protein synthesized by melanoma cells. This compound can be quantitatively separated from gp70 and it is not known if the two are related. Immunological and chemical characterization continues. Possible lymphokine production in stimulating tumor cell survival and growth is being investigated. Preliminary experiments indicate that supernatants from mitogen-activated lymphocytes that possess lymphokine activity in L-cell assays will replace lymphocytes in stimulating melanoma cell growth, suggesting lymphokines may be involved in promoting growth and survival of malignant cells. In other work results suggest that tumor cells can induce the solubilization of an endothelial basement membrane-like matrix, but the degradation requires endothelial cells and may be mediated by endothelial cells stimulated by tumor cell interactions. Variants and clones of the 13762 adenocarcinoma line have been selected to compare tumor and host properties important in mammary tumor metastasis. The reputed role of fibronectin in mammary tumor metastasis was studied using parental clones of high and low metastatic potential and cloned lines for lung metastasis. Preliminary results indicate fibronectin may not play a direct role in metastasis but may be important in determining the location of secondary tumor spread.

Significance to Cancer Research: A leading cause of death due to cancer is tumor metastasis, but little is known concerning the tumor and host properties important in metastatic tumor spread. Detailed information on the life-threatening, highly metastatic cell variants that exist in the primary tumor at low frequencies is necessary to develop suitable detection and therapeutic procedures to prevent metastasis.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Specific and Nonspecific Immunotherapy as an Adjuvant to Chemotherapy

Principal Investigator: Dr. Frederick Eilber
Performing Organization: University of California
City and State: Los Angeles, CA

Contract Number: NO1-CB-53941
Starting Date: 6/16/75 Expiration Date:

Goal: To determine if post-surgical adjuvant chemoimmunotherapy is superior to adjuvant chemotherapy in preventing disease recurrence in patients with highly malignant skeletal or soft-tissue sarcomas.

Approach: Patients with osteosarcoma or grade III soft-tissue sarcoma (rhabdomyosarcoma, synovial cell or anaplastic sarcoma) following complete surgical excision of their tumor are randomized between chemotherapy--adriamycin, high-dose methotrexate and citrovorum rescue--and identical chemotherapy plus immunotherapy consisting of BCG and allogeneic tissue culture sarcoma tumor cell vaccine. Chemotherapy is given for one year; the immunotherapy is given for the same year plus an additional year.

Progress: A total of 93 patients have entered this study, 50 who are receiving adjuvant chemotherapy and 43 who are receiving adjuvant chemoimmunotherapy. Of these 93, 36 have Stage I osteogenic sarcoma.

At this time no definite conclusion can be made as to whether adjuvant chemoimmunotherapy is superior to adjuvant chemotherapy alone in these patients. However, in Stage I skeletal sarcoma, a 30% incidence of metastases has been observed in patients who received chemoimmunotherapy compared to a 50% incidence of metastases in those who received chemotherapy alone.

Changing patterns of delayed cutaneous hypersensitivity to skin test antigens are being studied to determine whether there is any correlation with clinical course.

Significance to Cancer Research: The significance to this project has been to reduce disease recurrence and to prolong survival in a group of diseases that was previously rapidly fatal. Since the chemotherapy is discontinued after one year, it will be of major importance to determine if those patients who continue to receive immunotherapy will have a more lasting disease-free status than those patients receiving the chemotherapy alone.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $100,000

CONTRACT RESEARCH SUMMARY

Title: Human Melanoma: Evaluation of BCG Immunotherapy of Patients Without Detectable Disease After Removal of Tumor-containing Lymph Nodes

Principal Investigator: Dr. Donald L. Morton
Performing Organization: University of California
City and State: Los Angeles, CA

Contract Number: NO1-CB-64076
Starting Date: 8/8/74 Expiration Date: 10/07/79

Goal: To determine whether immunotherapy with BCG alone or BCG combined with tumor cell vaccine will decrease recurrence rate or prolong survival in melanoma patients with metastases to regional lymph nodes.

Approach: Patients are randomized following surgical lymphadenectomy into 3 groups receiving different postoperative therapy: one group receives no additional therapy, one receives BCG, and one group receives BCG and allogeneic tumor cell vaccine.

Progress: Fifty-two patients with Stage I disease have been admitted to this trial. Although 3 of the 4 patients who have recurred are in the control group, the number of recurrences remains too small for any statistical significance.

One hundred forty-nine (140 evaluable) patients with Stage II disease have been admitted to the study. There was no significant difference in recurrence rates between the immunotherapy and control patients. Patients receiving BCG immunotherapy who recurred are surviving significantly longer than those in the control group or those receiving BCG and tumor cell vaccine, which may be partially explained by a different pattern in the anatomical site of recurrence observed in the BCG group when compared to the other two groups.

Immunological monitoring studies are under way to try to determine whether any of the tests studied have value in predicting prognosis and assessing response to therapy in these patients.

Significance to Cancer Research: If the results of this study continue to develop significance as more patients are entered into the study, we can conclude that immunotherapy should become standard therapy for melanoma patients with lymph node metastases.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $135,784

CONTRACT RESEARCH SUMMARY

Title: Cell-mediated Reactivity of Normal Persons to Human Tumor-associated Antigens

Principal Investigator: Dr. Mitsuo Takasugi
Performing Organization: University of California
City and State: Los Angeles, CA

Contract Number: NO1-CB-74133
Starting Date: 9/16/77 Expiration Date: 9/15/79

Goal: Investigate the significance of the fact that effector cells from healthy individuals and cancer patients are normally cytotoxic to cultured human tumor cells.

Approach: Examine the level and specificity of the reaction with effector cells isolated from healthy people and patients; study the level of cells bearing Fc receptors in the circulation and how these levels are affected by cancer.

Progress: This contract is completing its second year of studies on natural cell-mediated cytotoxicity (NCMC). Selective NCMC has been detected against EBV- and HSV-associated antigens, HLA and natural target antigens. Using two-dimensional testing and two-way interaction analysis, specificity in NCMC or ADCC is detected. A third dimension is added to study modulation of NCMC by antibodies; ADCC and NCMC can be examined concurrently in this way. The importance of the Fc receptor on effector cells in the modulation of NCMC was observed in a model SRBC target system. Arming of effector cells with anti-SRBC allowed detection of cytotoxicity against SRBC but reduced NCMC and ADCC by the same effector cells, indicating its effect on Fc-Fc receptor interaction. Two factors released by lymphocytes appear to regulate the effector cell: NAF enhances and NIF suppresses activity. The co-existence of these two lymphokines provides a sensitive control of NCMC activity. NCMC is a specific phenomenon directed by antibodies working in conjunction with effector cells. IB activity may be influenced by antibodies and by factors which control the level of activity by the cells.

Significance to Cancer Research: It appears that natural cell-mediated cytotoxicity is a cell-mediated cytotoxic system whose specificities are directed by antibodies in the circulation. A better understanding of these processes and how cancer affects them is of great significance.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $74,282

CONTRACT RESEARCH SUMMARY

Title: Production of Monospecific Antibodies Against Human Malignant Lymphoma and Leukemia Tumor-associated Antigens

Principal Investigator: Dr. Ivor Royston
Performing Organization: University of California
City and State: San Diego, CA

Contract Number: NO1-CB-84250
Starting Date: 9/30/78 Expiration Date: 11/29/80

Goal: To produce xenogeneic monospecific antibodies to human lymphoma and leukemia associated antigens.

Approach: Attempts will be made to fuse antibody-producing cells of mouse origin with mouse myeloma cells in order to produce hybrid cell lines capable of continuous production of monospecific antibodies against tumor-associated antigens in vitro. Two different hybridization techniques will be compared. The specificity of the presumed monoclonal antibodies and the pattern of antigenic tumor-specific determinants on malignant lymphoid cells will be evaluated.

Progress: The investigator has succeeded in producing an established hybridoma cell line which continuously secretes monoclonal antibody directed against a human T-cell leukemia. This antibody, designated T-101/1, is cytolytic in the presence of rabbit complement and appears to detect a T-cell differentiation antigen found on cells of thymic origin. The potential use of this reagent in eliminating T cells from lymphoid populations is being explored.

In work done to compare two techniques of obtaining cloned hybridoma cell lines, results indicate that it is possible to prepare a single cell suspension from a 1 mm^3 spleen fragment and to fuse these cells with a mouse myeloma line to produce a hybrid cell line, and secondly that monoclonal antibody-producing spleen fragment cultures can be isolated.

Significance to Cancer Research: Large-scale production of monospecific anti-tumor cell antibodies produced in vitro may have significant application to immunotherapy and immunodiagnosis.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Characterization of Antigen-binding T-cell Receptors

Principal Investigator:	Dr. Joel W. Goodman
Performing Organization:	University of California
City and State:	San Francisco, CA
Contract Number:	NO1-CB-74178
Starting Date:	9/16/77
Expiration Date:	9/15/79

Goal: Purify T cell-derived antigen-binding receptors; evaluate new procedures for detection of antigen-binding T cells.

Approach: Use the response of A/J mice to chemically defined antigen (p-azobenzenearsonate) for these studies.

Progress: Reasonable progress has been made on 3 of the 4 aims of the contract workscope. Three procedures have been developed to isolate large numbers of cells of specific antigen-binding capacity. The first is to remove B cells from immune spleen cell populations using anti-Ig coupled to plastic dishes. This is followed by specific absorption to ABA-homologous Ig of the remaining T cells with ultimate recovery of the bound T cells by gentle pipetting in the cold. A second procedure involves in vitro blast formation of preimmunized spleen cells with isolation of the blasts on discontinuous BSA gradients. The third and final procedure is the development of a T-cell hybrid capable of specifically binding ABA which can be grown in vitro.

Preliminary studies on the characterization of T-cell antigen-binding receptors are also reported. Here the technique is in vitro labeling of cells with ^{35}S-methionine followed by lysis in non-ionic detergent and affinity chromatography on ABA-sepharose with hapten-specific elution. Components of approximately 90,000, 60,000, and 30,000 have been identified in extracts of spleen cells with an additional component of 140,000 on in vitro blasts.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $87,963

CONTRACT RESEARCH SUMMARY

Title: Studies of Immune Stimulants in Patients Receiving Radiation Therapy

Principal Investigator: Dr. William M. Wara
Performing Organization: University of California
City and State: San Francisco, CA

Contract Number: NO1-CB-64004
Starting Date: 6/1/76 Expiration Date: 5/31/80

Goal: To document the immunosuppression which occurs following radiation therapy and to determine if a therapeutic advantage can be achieved by the addition of immunotherapy (thymosin) to the conventional treatment given for squamous cell carcinoma of the head and neck region and the esophagus.

Approach: Both groups of head and neck and esophageal cancer patients will receive radiation therapy and/or surgery as treatment. Patients will then be randomized to receive thymosin or placebo in addition to standard treatment. All patients will be monitored with immunologic tests to measure antibody-mediated and cell-mediated immunity at regular intervals. The data will be analyzed for local disease recurrence, metastatic disease, and survival difference in the patient groups.

Progress: Results on 103 patients studied indicate the expected immunosuppression after irradiation. Preliminary disease-free interval data for the head and neck cancer patients indicate no significant difference between the thymosin and placebo groups. In a subgroup of patients results show that at three months post-therapy there is a significant difference in enhancement of the MLC response in the thymosin-treated patients and a slight but not significant trend toward increased PHA stimulation.

Temporary unavailability of thymosin compromised entry into that treatment group for a while.

Significance to Cancer Research: This study will determine the significance of immunosuppression caused by irradiation in head and neck and esophageal cancer patients and whether the effect of immunotherapy (thymosin) in reversing this immunosuppression has any impact on disease-free interval, survival, and local control of cancer.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $111,748

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy of Murine Leukemia Using Syngeneic Hybrid Cells

Principal Investigator: Dr. Edward P. Cohen
Performing Organization: University of Chicago
City and State: Chicago, IL

Contract Number: NO1-CB-74195
Starting Date: 9/30/77 Expiration Date: 9/29/79

Goal: To evaluate the efficacy of syngeneic hybrid cells in the treatment of spontaneously occurring and passively transferred murine leukemia.

Approach: Somatic hybrids of murine leukemia and LM cells, a sustained mouse tissue culture line, are rejected by syngeneic mouse recipients. Mice rejecting such hybrid cells resist in part what are otherwise invariably lethal challenges of the leukemia cells used as one parent in forming the hybrid. Immunity in resistant animals is directed at least in part toward a tumor-associated antigen of parental and hybrid cells.

Progress: The immunotherapeutic properties of ASL-1XLM(TK)-hybrid cells after 18 months in continuous culture have changed. Animals receiving leukemic cells followed by hybrid cells died in approximately the same period as animals receiving leukemic cells alone. Although the immunotherapeutic properties were lost during long-term culture, immunoprotective properties were maintaned.

The mean chromosome number of chromosomes of ASL-1XLM(TK)-hybrid cells after prolonged culture in vitro diminished from 85 $\pm$ 3 to 60 $\pm$ 5.

Studies revealed that the relative quantities of tumor-associated antigens expressed by hybrid cells after long-term culture are approximately 22-fold less than that expressed by mutamycin-C-treated ASL-1 cells.

Significance to Cancer Research: The overall objective of this project is to develop a useful therapeutic adjunct to the treatment of human leukemia. Hybrid cells lose their oncogenic potential in immunocompetent hosts, but retain the surface characteristics of neoplastic cells. They become effective immunizing agents.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Characterization of Antigen-binding T-cell Receptors

Principal Investigator: Dr. Frank W. Fitch
Performing Organization: University of Chicago
City and State: Chicago, IL

Contract Number: NO1-CB-74149
Starting Date: 9/19/77 Expiration Date: 9/29/79

Goal: Characterize antigen receptors on rat T cells which are specific for rat alloantigens.

Approach: Preparation of monospecific anti-idiotypic antisera; development of continuous cell lines of rat lymphoid cells which secrete homogeneous antibody reactive with rat alloantigens; screening of various rat T-cell populations for the presence of relevant idiotypic receptors.

Progress: This contract is completing its second year of work. Recent focus has been on the use of characterized idiotypic antisera to test for the presence of the relevant idiotypic receptor on rat T-cell populations and to expand idiotypic positive T-cell populations through in vitro culture techniques. Anti-idiotypic antisera have been prepared in rats and rabbits by immunization with purified hybridoma antibodies. These antisera were detected using a solid phase radioimmunoassay and were used to detect T-cell receptors. Various modifications in tissue culture techniques have been evaluated to determine conditions under which continuous cloned lines of alloreactive T cells might be obtained.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $86,883

CONTRACT RESEARCH SUMMARY

Title: Reagents for Characterization of Human Cell Subpopulations

Principal Investigator: Dr. Robert Hunter
Performing Organization: University of Chicago
City and State: Chicago, IL

Contract Number: NO1-CB-84287
Starting Date: 9/30/78 Expiration Date: 9/29/79

Goal: Development of new reagents for the characterization of subpopulations of human cells important to the immune response or detection and characterization of new subpopulations of these cells.

Approach: Use lymphoid cells from mice immunized with human lymphoid cells in hybridization experiments to produce antibodies which react with human lymphocyte subclasses. Produce and characterize appropriate antibodies and determine their ability to define subsets of lymphocytes.

Progress: In the initial phase of the contract monoclonal antibodies against human lymphocytes have been produced in three cell hybridization experiments. From these hybridizations 16 clones have been selected for further study. These all show strong cytotoxicity against the MOLT-4F human T-lymphocyte line and variable cytotoxicity against other human lymphocyte lines and peripheral blood lymphocytes. Four patterns of cytotoxicity have been identified with these cell lines, implying that the antibodies recognize different antigenic determinants in human lymphocytes.

Significance to Cancer Research: The development of reagents for characterizing subpopulations of human cells important to the human response may facilitate analyses and manipulation of the immune response to human tumors.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $83,692

CONTRACT RESEARCH SUMMARY

Title: Collection of Serial Serum Samples from Cancer Patients

Principal Investigator: Dr. Robert E. Gerner
Performing Organization: University of Colorado
City and State: Denver, CO

Contract Number: NO1-CB-74161
Starting Date: 9/30/77 Expiration Date: 12/31/78

Goal: Collect serial serum specimens from patients with malignant and benign diseases and "normal" individuals for NCI to evaluate immunodiagnostic tests for cancer.

Approach: Collect serial serum specimens on cancer patients before therapy and every three months following and on benign diseases and control patients. Samples are stored at -70°C in 1 ml aliquots; complete clinical data are kept on patients; a minimum of 300 patients will be entered into the study.

Progress: We have purchased and obtained all necessary equipment and clinical forms to perform the study. We have interviewed 150 individuals (these include those with various malignant and benign disease plus normals) of which 75 have consented to participate in this study. From these 75 individuals, a total of 1,190 1 ml vials of serum have been processed and stored at -70°C. Complete clinical data have been obtained and recorded on all of these patients.

Significance to Cancer Research: The significance of this portion of the total project is to collect, process, and make available to other investigators serial serum samples from patients with various malignancies in an attempt to develop immunodiagnostic tests for cancer.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Cryopreservation of Human Monocytes for Use in Immunologic Studies

Principal Investigator: Dr. Roy S. Weiner
Performing Organization: University of Florida
City and State: Gainesville, Florida

Contract Number: NO1-CB-74131
Starting Date: 9/30/77 Expiration Date: 9/29/79

Goal: To isolate human monocytes from peripheral blood and develop methods for their adequate cryopreservation.

Approach: Develop methods of collection and isolation of peripheral blood monocytes that would be applicable to normal subjects and to patients with cancer. Develop optimum techniques for cryopreservation and define the functional characteristics of cryopreserved cells. Evaluate changes in monocyte structure and function occurring in patients with cancer.

Progress: This contract is in its second year. The contract workscope calls for optimization of separation and cryopreservation techniques for monocytes and to do functional studies on these cells from cancer patients and normal subjects. A microassay for hexose monophosphate shunt activity which permits use of 10^6 monocytes per tube has been developed. A monocyte chemotaxis assay has been established and preliminary runs indicate the cryopreserved monocytes retain their functional activity. An ^{125}I-labeled IUdR cytotoxicity assay has been developed using P815 murine mastocytoma cells as targets and mouse peritoneal macrophages as cytotoxic effector cells. Studies of monocyte collection by the cell separator indicated that 80% or more of the monocytes present in a unit of peripheral blood could be harvested in the Haemonetics buffy coat. Studies on cryopreservation of monocyte function in mononuclear cell preparations cryopreserved in DMSO showed that hexose monophosphate shunt activity, histochemical staining for nonspecific esterase myeloperoxidase, and chemotactic activity could be preserved. Studies have begun of cytotoxicity of human monocytes against murine P815 mastocytoma cells. It was shown that cryopreserved cells, when activated by lymphokine or lipopolysaccharide, could exert a cytotoxic function against the target cells but native (unactivated) peripheral blood monocytes were not cytotoxic.

Significance to Cancer Research: We have made significant progress in collection procedures that are compatible with sequential samplings of large numbers of monocytes from patients with minimal loss of blood. We have facilitated the testing of monocyte function as it may change with anti-tumor therapy.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $99,258

CONTRACT RESEARCH SUMMARY

Title: Cells Involved in Immune Response to Tumors

Principal Investigator: Dr. Pekka J. Hayry
Performing Organization: University of Helsinki
City and State: Helsinki, Finland

Contract Number: NO1-CB-64032
Starting Date: 6/28/76 Expiration Date: 6/27/79

Goal: To analyze the origin, type, and receptor specificity of allograft-infiltrating cells, as well as their functions in the rejection process and later to perform similar studies with syngeneic tumor grafts.

Approach: The following approaches have been used: (1) mouse sponge matrix allografts (SMG); (2) human and rat kidney allografts; and (3) human cancers. In SMG the infiltrating cells were recovered by compression, the rest of the grafts were disaggregated by collagenase-DNAse, and the infiltrating cells were separated from tumor or parenchymal cells by 1 g velocity sedimentation.

Progress: The SMG is first infiltrated by T killer cells, later also cytotoxic non-T lymphocytes and monocytes participate in the infiltrate. Most of the infiltrating cells, including cells of the monocytic lineage, carry idiotypic receptors to the graft donor antigens. A mixed population of cells, including (in this order of magnitude) monocytes/macrophages, T lymphocytes, non-T lymphocytes, B cells, plasmablasts/plasma cells, and T lymphoblasts, infiltrate rat and human kidney allografts. The infiltrating cells are far more cytotoxic than blood or (in rat) spleen lymphocytes to donor-derived lymphoid target cells. Variable inflammatory population consisting of (in this order of magnitude) IgG-FcR positive monocytes/macrophages, null lymphocytes, T and B lymphocytes but no or very few blast and plasma cells infiltrate human cancers. Even when blood leukocytes are cytotoxic to the patient's own cancer cells, the infiltrating cells are not. The sequence of appearance of the different types of killer cells in allografts, and the reason(s) for the lack of cytotoxicity within cancers are under analysis.

Significance to Cancer Research: Elucidates the in situ cytotoxicity mechanisms in the anti-allograft and anti-cancer immune response.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Specificity of Antigen-binding Receptors on T Cells

Principal Investigator: V. E. Olavi-Makela
Performing Organization: University of Helsinki
City and State: Helsinki, Finland

Contract Number: NO1-CB-43923
Starting Date: 6/1/74 Expiration Date: 5/31/79

Goal: To find out whether the receptor antibodies that mediate recognition by T lymphocytes are controlled by the Mendelian genes that control specificity of circulating antibody molecules (V_H genes).

Approach: To determine whether T lymphocytes of an individual carry the V_H gene controlled idiotypes that are carried by Ig-molecules of this individual.

Progress: Before the present contract year, we had found a Mendelian polymorphism in mouse antibodies against three haptens (NP, ABA-HOP and NBrP). For example, anti-NP antibodies of C57BL mice have systematically different V_H regions than BALB antibodies against this hapten. We had prepared anti-idiotypic reagents against the products of the V_H NP gene of C57BL and against the V_HABA-HOP gene of A/J. In collaborative work with Rajewsky, we had found that nylon-adherent T cells have antibodies carrying the idiotypic and fine-specificity characteristics determined by the (Ig) genotype of the mouse. During the report period, we have found two new V_H genes: in antibodies to cloxacillin and oxazolone. We have prepared anti-idiotypic reagents against the anti-oxazolone antibody of BALB/c. We have demonstrated a hapten-specific T-lymphocyte help with two of these haptens, NP and oxazolone. The next step was to demonstrate that idiotypic antibody on the T cells is functionally relevant: lethally irradiated mice received BSA-immunized B cells and NP-immune or nonimmune T cells (nylon wool) plus NP-BSA challenge. The former group developed four times as many BSA PEC as the latter, and the increase was inhibited. Recipients received 0.1 ml of mouse anti-idiotype serum.

Significance to Cancer Research: T cells play an important role in the body defenses against cancer. Elucidation of their mode of recognition is important.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Reagents for the Characterization of Human Cell Subpopulations

Principal Investigator: Dr. Marius Teodorescu
Performing Organization: University of Illinois
City and State: Chicago, IL

Contract Number: NO1-CB-84268
Starting Date: 9/30/78 Expiration Date: 9/29/80

Goal: Development of new reagents for the characterization of subpopulations of human cells important to the immune response or detection and characterization of new subpopulations of these cells.

Approach: Determine by in vitro tests the function in immune response of lymphocyte subpopulations isolated by specific absorption of human lymphocytes to a bacterial monolayer fixed on a glutaraldehyde-fixed gelatin bed.

Progress: This contract is in its first year of work, studying lymphocyte subpopulations isolated by bacterial monolayer. Subpopulations described are T_1 through T_4, with NK cells in T_4. Possible suppressive activity in T_1 or T_2 has been investigated; these populations separate or together have relatively poor NK activity. With the addition of T_2 or T_1T_2 cells to T_3T_4 cells, an insignificant decrease in NK activity is seen. T_1 cells alone had no effect. However, when T_2 or T_1T_2 cells are added to whole PBL, a significant decrease in NK activity of greater than 50% was found. Other experiments showed that this effect was not due to simple dilution of PBL cell activity. These preliminary results are strongly suggestive of T_2 cells being NK suppressors, but further studies will have to be done. A Chang hepatoma cell line has been grown and rabbit antigen has been raised in preparation for ADCC studies on these subpopulations.

Significance to Cancer Research: The development of reagents for characterizing subpopulations of human cells important to the immune response may facilitate analyses and manipulation of the immune response to human tumors.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Purification of Human Tumor-associated Antigens

Principal Investigator: Dr. David Gold
Performing Organization: University of Kentucky
City and State: Lexington, KY

Contract Number: NO1-CB-84259
Starting Date: 9/30/78 Expiration Date: 9/29/79

Goal: Purification, isolation, and identification of tumor-associated antigens.

Approach: Extract and purify colonic mucoprotein antigen (CMA) from normal colon and colonic adenocarcinomas; develop a radioimmunoassay and immunohistochemical procedures for detection of CMA; investigate the potential of CMA as a tissue- or tumor-specific marker.

Progress: This contract is in its first year and is concerned with the isolation of colonic mucoprotein antigen (CMA) and development of a radioimmunoassay for detection of this organ-specific antigen. The original plan called for emphasis on purification of the antigen during the first year and this is well under way, good purification procedures having been developed. CMA has been purified from one normal colon and five primary colon carcinomas in amounts ranging from 5 to 31 mg. Attempts to purify CMA from GW39 and LS174T (colon carcinomas grown as xenogeneic grafts in hamsters and nude mice) have given poor antigen yields, considering the large amount of mucin present in the starting homogenate. Also the purified antigens did not give precipitin reactions in immunodiffusion with rabbit anti-normal tissue CMA antiserum whereas the starting homogenate did. Methods are being worked out to isolate mucin from GW39 in the highest possible yields. Rabbit anti-normal tissue CMA antiserum has been prepared and, at the level of sensitivity obtained with immunodiffusion, this antiserum appeared to be colon specific. The antiserum is currently being absorbed in anticipation of utilizing more sensitive techniques (immunoperoxidase). Antisera are also being prepared against other antigen preparations as well.

Significance to Cancer Research: Purification of tumor-associated antigens for use in diagnostic testing and evaluation of immunologic assays, particularly radioimmunoassays, is highly relevant to cancer immunodiagnosis.

Project Officer: Ms. Judith Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $94,491

CONTRACT RESEARCH SUMMARY

Title: Immunohistochemical Studies of Tumor-associated Antigens

Principal Investigator: Dr. F. James Primus
Performing Organization: University of Kentucky
City and State: Lexington, KY

Contract Number: NO1-CB-84257
Starting Date: 9/18/78 Expiration Date: 9/17/79

Goal: To isolate and characterize cancer-related markers in or on tumor cells by immunohistochemical techniques and describe their relationship to the discrimination of neoplastic cells from normal cells.

Approach: Studies will be performed using colon carcinoma antigen III, CSAp, beta HCG, Regan isoenzyme, and major blood group antigens in an attempt to discriminate between malignant and nonmalignant cells and to determine whether immunocytochemical methods can accurately detect metastatic spread in regional nodules.

Progress: The intent of this contract is to use immunohistochemical tests to discriminate between malignant and nonmalignant cells, to assess the use of such tests to detect micrometastases, and to assess the use of such tests to monitor tumor progression and regression when applied to either biopsy material or serum. Progress during the first six months of the study includes the standardization of tests for CEA-III, CCA-III (colon carcinoma antigen III), CSAp (colon specific antigen p), blood group antigens A and B, and beta-HCG. In all cases, an indirect antibody-conjugated immunoperoxidase test was found superior to a previously studied triple-bridge procedure. Purified antibodies prepared by negative immunoabsorption with possible cross-reacting materials and checked for specificity by positive immunoabsorption are used. One retrospective study where the immunoperoxidase technique for CEA was used indicated that this test was as accurate as histopathology in detecting micrometastatic spread with no false positive or false negative results recorded.

Significance to Cancer Research: The immunohistochemical studies of well-known or well-defined tumor-associated antigens in tissue sections may be useful in the diagnosis or prognostic evaluation of cancer.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $70,056

CONTRACT RESEARCH SUMMARY

Title: Diagnostic Application of Human Tumor-associated Antigen

Principal Investigator: Dr. Robert Humphreys
Performing Organization: University of Massachusetts
City and State: Worcester, MA

Contract Number: NO1-CB-64072
Starting Date: 6/30/76 Expiration Date: 6/29/79

Goal: To identify and isolate membrane proteins from leukemic cells in order to raise rabbit antibodies which may be used to diagnose biologic subsets of leukemias and lymphomas by cytotoxic or immunofluorescent assays.

Approach: Membrane proteins from leukemic cell lines are examined by [^{35}S]-methionine, autoradiographic techniques in SDS-polyacrylamide slab gel electrophoresis. T- and B-specific proteins are identified, and are then isolated by traditional protein chemical techniques. Antisera to these proteins are assayed for their specificity for leukemia or for subsets of leukemias.

Progress: p23,30 antigen from lymphoid malignancies has been isolated by raising goat anti-p23,30 serum. The distribution of p23,30 antigen on subsets of normal cells, after mitogenic or allogeneic stimulation, and on leukemic cells and lymphomas is being studied. Other non-HLA-A, -B and -D antigen proteins have been isolated from spleens infiltrated with lymphoid malignancies and antisera to these partly purified, and in some instances homogeneous, proteins are being raised. EBV transformed B-cell lines from normal individuals appear similar in their patterns to Burkitt's lymphoma cells, although a few proteins appear uniquely on some cell lines. T-lymphoblast cell lines are substantially different from B-cell lines in the appearance of their membrane protein patterns. That the heterogeneity in the cell lines was not the result of variability among outbred humans was demonstrated by identity of patterns from PHA-stimulated lymphocytes from laboratory personnel.

Significance to Cancer Research: The identification of biological subsets of leukemias permits correlation to the clinical course. These assays may in time affect the selection of chemotherapeutic protocols based on the identification of biological subsets of cancers, or the antisera may be used to modify the immune response in patients with cancer.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Suppressor Monocytes in Cancer Patients

Principal Investigator: Dr. Norman T. Berlinger
Performing Organization: University of Minnesota
City and State: Minneapolis, MN

Contract Number: NO1-CB-74160
Starting Date: 9/16/77 Expiration Date: 8/15/79

Goal: To study the effects of suppressor monocytes on cellular immune functions in cancer patients and the relevance of these effects to the clinical course of the disease.

Approach: To study how frequently patients with epidermoid cancer of the head and neck show evidence of circulating suppressive monocytes and how the presence, absence, disappearance, or reappearance of these cells correlates with prognosis and the clinical course of the disease.

Progress: Nearly 50 patients with this cancer have been studied to date. The majority of them show deficits of cellular immunity, many of the magnitude seen in congenital immunodeficiency diseases. Although follow-up so far has been short, it appears that patients with deficient cellular immunity as measured in mixed leukocyte culture seem to have poorer prognoses. In most of these patients the deficient cellular immunity seems to be secondary to a population of regulatory cells which inappropriately exert a suppressive effect on the defensive functions of T-lymphocytes. These regulatory cells appear to be monocytes and to inhibit T-lymphocyte blastogenesis. The identity of this cell as a monocyte has been suspected by our experiments that eliminate patient monocytes from mixed cell cultures with the result of increased lymphocyte blastogenesis, and from experiments that return patient monocytes to these cultures with the effect of suppressed lymphocyte blastogenesis.

Significance to Cancer Research: A critical quantification of cellular immunity in the cancer patient could help identify those patients who are at high risk for doing poorly with their disease. An understanding of the nature of the deficient immune responses could allow the development of rational immunotherapeutic modalities.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Immunologic Mechanisms of Cattle

Principal Investigator: Dr. Charles Muscoplat
Performing Organization: University of Minnesota
City and State: St. Paul, MN

Contract Number: NO1-CB-84244
Starting Date: 9/30/78 Expiration Date: 9/29/79

Goal: Perform in vitro studies of immunologic mechanisms in cattle in conjunction with ongoing immunoprophylaxis studies to gain information on the possible influence of immunity on carcinogenesis.

Approach: Perform in vitro studies on the cellular and humoral events of the bovine immune system on tissues provided by NCI from experimental animals involved in a study of immunoprophylaxis of bovine ocular squamous cell carcinoma to determine whether correlative changes in immune response can be detected during the process of cancer development and, if so, to determine the immunologic mechanisms involved.

Progress: Progress during the four months since this contract was awarded is in part due to the techniques and materials made available to Dr. Muscoplat by Dr. Kleinschuster, principal investigator of a contract with Utah State University. Methods have been developed by Dr. Kleinschuster to cryopreserve bovine mononuclear cells, and Dr. Muscoplat has recovered and identified T cells, B cells, and monocytes in good yield. Mononuclear cells have been obtained from cattle at different stages of malignant disease (ocular carcinoma). Cell lines of the tumors have been established at Utah and will be made available to Dr. Muscoplat who has shown that the cultured cells can be used as targets for immunologic studies.

Project Officers: Dr. Herbert Rapp and Ms. Judith Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $84,000

CONTRACT RESEARCH SUMMARY

Title: Reagents for the Characterization of Human Cell Subpopulations

Principal Investigator: Dr. Noel Warner
Performing Organization: University of New Mexico
City and State: Albuquerque, NM

Contract Number: NO1-CB-84288
Starting Date: 9/30/78 Expiration Date: 9/29/79

Goal: Development of new reagents for the characterization of subpopulations of human cells important to the immune response or detection and characterization of new subpopulations of these cells.

Approach: Develop specific heteroantisera against defined populations of human lymphoid cells stressing nonmalignant populations, including Tγ, Tμ and T null cells, and other T-cell preparations, as well as B-cell populations defined by various available cell surface markers, and macrophage subpopulations.

Progress: This project is in its first year, and the progress report essentially represents three months of work after technical personnel were trained and techniques developed. Work has focused primarily on the development of a heteroantiserum which appears to identify a suppressor cell subpopulation. The immunogen has been mononuclear cells from a patient with hypogammaglobulinemia (CVH). The antibody is not B- or T-cell specific and reacts more strongly with sera from patients with CVH than from normal controls. Reactivity is demonstrable with two of three B-cell lines tested and one T-cell line. The question is raised whether the antiserum may be directed against Ia-like (DR) antigen. One explanation might be that this is an anti-DR allotype serum and this allotype might identify a specific antigen abnormality on cells from patients with CVH. This laboratory has preliminary data on patients with CVH, suggesting they may have an abnormal Ia-like antigen on a significant proportion of their T cells. In two assays the antiserum appears to identify suppressor cells.

Significance to Cancer Research: The development of reagents for characterizing subpopulations of human cells important to the immune response may facilitate analyses and manipulation of the immune response to human tumors.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $88,398

CONTRACT RESEARCH SUMMARY

Title: Genetic Control of Susceptibility to Cancer

Principal Investigator: Dr. Geoffrey Haughton
Performing Organization: University of North Carolina
City and State: Chapel Hill, NC

Contract Number: NO1-CB-74182
Starting Date: 9/30/77 Expiration Date: 9/29/79

Goal: Investigate the finding that a polymorphic gene or genes, linked to the H-2 complex in mice, determines susceptibility to primary sarcoma induction following neonatal injection of RSV-containing chicken tumor material.

Approach: Investigate the mechanism of action of the genes to determine whether differences in immune response to tumor antigens are involved. Explore the finding that both dominant and recessive resistance exist. Test for immune surveillance and immune stimulation.

Progress: This contractor is completing his second year on this project. Since the last progress report was submitted, it has been demonstrated by immunosuppression and by cell transfer studies that separate components of the immune response serve to stimulate and to suppress the development of primary Rous sarcoma in neonatally infected mice. The nature of the cell population and the timing of their administration profoundly affect whether stimulation or suppression of tumor development results. The contractor has shown that offspring of mothers actively immunized against Rous sarcoma are protected against primary tumor development following neonatal injection relative to offspring of non-immunized mothers. On the other hand, the offspring of passively immunized mothers are relatively more susceptible to tumor induction than normal controls. Thus an immunostimulatory or enhancing role for tumor-specific antibody is established, transferred to babies either by transplacental passage or in the milk.

Significance to Cancer Research: We have demonstrated a clear link between genetic control of tumor susceptibility and genetic control of tumor-specific immune responsiveness. Preliminary data suggest that different components of the immune response stimulate the growth of and eliminate tumors.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $82,731

CONTRACT RESEARCH SUMMARY

Title: Selective Depletion of Mononuclear Phagocytes In Vivo

Principal Investigator: Dr. Stephen Russell
Performing Organization: University of North Carolina
City and State: Chapel Hill, NC

Contract Number: NO1-CB-84271
Starting Date: 9/30/78 Expiration Date: 9/29/81

Goal: Development of methods for the selective in vivo destruction or sustained functional inactivation of mononuclear phagocytes.

Approach: Produce specific antimononuclear phagocyte globulin by selective absorption and assess the effect of these reagents on mononuclear phagocytes in vivo using "immune" granuloma formation in presensitized mice and spontaneous regression of Moloney sarcomas in mice as model systems.

Progress: This contract is in its first year and is concerned with producing antibody that will deplete or functionally neutralize mononuclear phagocytes in vivo. In the initial contract period, a continuously cultured macrophage line that can be activated in the same way as normal peritoneal macrophages has been discovered. Immunizations for production of antimononuclear macrophage antisera have been initiated using macrophages from the various sources described in the workscope. The contractor has mastered the technique for liquid bone marrow culture and perfected techniques for generating and harvesting blood monocytes. Assay techniques are in place for evaluation of the antisera.

Significance to Cancer Research: Studies on the in vivo depletion of macrophages will aid in examining the roles of macrophages in tumor destruction.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy of Syngeneic Tumors in Wistar Rats

Principal Investigator: Dr. Robert W. Baldwin
Performing Organization: University of Nottingham
City and State: Nottingham, England

Contract Number: NO1-CB-64042
Starting Date: 6/18/76 Expiration Date: 6/17/79

Goal: To evaluate the relative effectiveness of bacterial immunostimulants including BCG and C. parvum for active specific immunotherapy and adjuvant contact therapy of transplanted rat tumors, especially those reviewed as being relevant models for human disease.

Approach: Two modalities of treatment are being evaluated in susceptible systems, such as carcinogen-induced rat sarcomas, to define optimum conditions. These procedures are then being used to determine their value in the treatment of more clinically relevant systems, e.g., transplants of spontaneous rat sarcomas and mammary carcinomas. In each system the effectiveness of therapy is being assessed against local tumor and metastatic deposits.

Progress: These investigators have reported the superior reactivity of Wellcome C. parvum and fresh suspensions of BCG (when compared with lyophilized preparations) in adjuvant contact therapy of a variety of rat tumors.

Active specific immunotherapy (tumor cell vaccine combined with BCG, MER or C. parvum) is effective in controlling growth of relatively small amounts of tumor. The effect is most pronounced on immunogenic tumors.

Significance to Cancer Research: In situ stimulation of host responses of both specific and nonspecific types by adjuvant contact therapy has potential for immunotherapy. This suggests that stimulation of nonspecific immunity may be effective for treatment of tumors in other organs and this is being evaluated with both animal and human lung tumors.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Detection of Tumor-specific Antigens in the Circulation

Principal Investigator: Dr. R. W. Baldwin
Performing Organization: University of Nottingham
City and State: Nottingham, England

Contract Number: NO1-CB-74167
Starting Date: 9/30/77 Expiration Date: 9/29/79

Goal: Investigate the diagnostic applicability of circulating tumor-associated antigens.

Approach: Follow the levels of circulating antigens during tumor growth in a variety of situations, attempting to relate measured levels of antigen to tumor size. A wide range of rat tumors (3-methylcholanthrene-induced sarcomas, hepatocellular carcinomas, and spontaneously arising mammary adenocarcinomas in Wistar rats) will be used.

Progress: This contract is completing its second year. Studies using a transplanted rat hepatoma (D23) show that serum-borne tumor antigen can be detected, using a membrane immunofluorescence inhibition test, well before subcutaneous or intramuscular tumor challenges become clinically detectable. Monitoring of serum levels of tumor antigen can be used to determine whether tumor will subsequently develop. Studies indicate that the release of tumor antigen into the circulation is a feature of viable tumor cells since serum-borne tumor antigen was not detected when radiation-attenuated hepatoma D23 cells were injected. A transplanted rat mammary adenocarcinoma (Sp4) model is being studied and is potentially useful as a model for human cancer because it arises naturally in the epithelial tissues, and undergoes metastatic spread to regional nodes. The pattern of tumor antigen release into circulation following subcutaneous implantation is comparable with that observed with D23. Progress has been slow in developing a cell-free radioimmunoassay and attempts are being directed toward development of a cell-binding assay. This assay seems to have sufficient sensitivity and reproducibility to be used for detecting serum levels of tumor antigen.

Significance to Cancer Research: These studies will establish in animal systems the value of monitoring circulating tumor antigen for diagnosis.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $58,780

CONTRACT RESEARCH SUMMARY

Title: Immunogenicity of "Spontaneous" Animal Tumors

Principal Investigator: Dr. Michael J. Embleton
Performing Organization: University of Nottingham
City and State: Nottingham, England

Contract Number: N01-CB-64009
Starting Date: 6/18/76 Expiration Date: 6/17/79

Goal: To determine (a) the frequency of expression of tumor-associated antigens capable of inducing host resistance in spontaneously arising tumors of an inbred rat strain, and (b) the influence of these antigens on tumor development.

Approach: Spontaneous rat tumors arising in a normal breeding population are transplanted into syngeneic hosts and their immunogenicity is assessed by immunization/challenge protection studies in vivo, carried out mainly during early transplant generations. The transplantation studies are supported by serological testing for antibodies reactive against tumor cell surface antigens.

Progress: More than 100 spontaneous tumors have been transplanted into syngeneic rats over a number of years, and immunogenicity tests have been completed with 24 of them. Rats were immunized by surgical removal of a growing tumor transplant or by treatment with attenuated tumor, followed by challenge with viable tumor cells. Immunity to challenge with cell doses growing in control rats was induced against 7 of the 24 tumors. In most cases the response was weak, but in some cases immunity approaching the levels experienced with chemically induced or virus-induced tumors was observed, this often being accompanied by a humoral antibody response to the tumor. In all cases, the responses detected were specific for individual tumors. The adjuvant agents BCG and Corynebacterium parvum were tested for their ability to increase resistance to spontaneous tumors, but they were effective only in conjunction with immunogenic tumors.

Significance to Cancer Research: Spontaneous tumors are more relevant to human tumors than are experimentally induced tumors. These studies therefore suggest that some human tumors could have specific antigens relevant for host immunity.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Immunoprophylaxis of Bovine Lymphosarcoma

Principal Investigator:	Dr. Simon Kenyon
Performing Organization:	University of Pennsylvania
City and State:	Philadelphia, PA
Contract Number:	NO1-CB-84243
Starting Date:	9/30/78
Expiration Date:	9/29/79

Goal: Study the immunoprophylaxis of bovine lymphosarcoma through administration of BCG cell wall vaccine by intravenous injection in a single dose as a possible guide for the control of analogous human disease.

Approach: Study the effect of the administration of a single intravenous dose of BCG cell wall vaccine on the extent and rate of development of lymphosarcoma in cattle injected with bovine leukemia virus at a stage prior to the development of discernible evidence of disease.

Progress: During the three months since the initiation of this study all animals have been acquired, matched, and divided into a treatment group and a control group. Treatment was given in April 1979. Methods are available for diagnosis of this primary autochthonous malignancy and for prognosis.

Project Officers: Dr. Herbert Rapp and Ms. Judith Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $151,988

CONTRACT RESEARCH SUMMARY

Title: Evaluation of Immunity Induced by Drug (DTIC)-mediated Tumor Antigens (DMTA)

Principal Investigator: Dr. Enzo Bonmassar
Performing Organization: University of Perugia
City and State: Perugia, Italy

Contract Number: NO1-CB-64054
Starting Date: 6/30/76 Expiration Date: 6/29/79

Goal: Studies concerning the origin and the properties of new tumor-associated transplantation antigens induced in cancer cells following treatment of tumor-bearing mice with drugs.

Approach: Xenogeneization of tumor cells in vivo by treatment of tumor-bearing hosts with dimethyltriazenoimidazole-carboxamide (DTIC). Utilization of the drug-mediated transplantation antigens for antitumor immunotherapy.

Progress: A line of L5178 from DTIC-treated mice was inoculated into immunosuppressed mice in high dilution, such that each mouse received only several cells. The outgrowing sublines were then tested for immunogenicity by determining the dose of whole-body irradiation needed to make recipients succumb to the lymphoma, and by using the sublines as stimulators and targets in crossed experiments in in vitro sensitization for CML. It was concluded that the sublines were immunogenic with shared but not identical antigenic profiles. Successful immunochemotherapy of mice bearing weakly immunogenic lymphoma can be attained using the following treatment schedule: (1) DTIC (for producing DMITI); (2) CY for producing "vacuum" in host's lymphoid system; (3) transfer of syngeneic lymphocytes (which proliferate and restore graft responsiveness against DTIC-induced DMITI); (4) BCNU chemotherapy (for producing antitumor effects in combination with anti-DMITI immune responses).

Significance to Cancer Research: The possibility of inducing new antigenic targets on cancer cells by treating the tumor-bearing host with drugs could open new perspectives in cancer immunotherapy.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy of C3H Murine Mammary Carcinomas

Principal Investigator: Dr. Bernard Fisher
Performing Organization: University of Pittsburgh
City and State: Pittsburgh, PA

Contract Number: NO1-CB-64044
Starting Date: 6/16/76 Expiration Date: 9/15/79

Goal: To improve growth inhibition of murine mammary carcinoma, obtained by combined immunotherapy (C. parvum; CP) and chemotherapy (cyclophosphamide; CY), by adding or substituting immunomodulators and chemotherapeutic agents, and by varying aspects of administration.

Approach: Since intratumor (i.t.) CP has shown superior effectiveness, its optimal dose will be determined. Effort will be aimed at manipulating therapy of a primary tumor to improve distant tumor control. Experiments will evaluate radiation or mitomycin-C plus CP following primary tumor removal. The combination will be used when primary tumors are removed or after i.t. immunotherapy. Studies will evaluate the effect of radiation and i.t. immunotherapy on distant tumor. Administration of NSSAs by i.t. and systemic routes simultaneously will be investigated. The effect of altering therapeutic regimens after the response to one regimen has been achieved will be studied.

Progress: BCG, C. parvum, bru-tel (an aqueous ether extract of Brucella abortus), and glucan were administered alone or in combination with chemotherapy, surgery, or radiation. Several routes of administration of the immunostimulants have been studied in tumor-bearing mice. Bru-tel and glucan augment CY effectiveness but not to the same extent as CP. Intratumoral treatment with CP resulted in inhibition or complete regression. Systemic CP given after tumor irradiation had little effect. Systemic CP and CY following 2000 R resulted in tumor growth inhibition, greater than that produced by any of the 3 agents alone or 2 in combination. Radiation of a tumor prior to CY and local CP therapy resulted in greater inhibition of tumor growth and survival. Tumors irradiated prior to chemoimmunotherapy responded uniformly to therapy in contrast to responses observed without radiation.

Significance to Cancer Research: Findings suggest an advantage to delaying primary tumor removal so that it may be used in multimodality therapeutic strategies to control metastatic disease. Administering immunostimulating agents directly into a primary tumor may increase host immune response and eliminate residual tumor, which chemotherapy alone is unable to control.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Immunoprevention of Malignant Tumors

Principal Investigator: Dr. Beverly Weeks
Performing Organization: University of South Carolina
City and State: Columbia, SC

Contract Number: NO1-CB-84242
Starting Date: 9/30/78 Expiration Date: 9/29/79

Goal: Determine whether, before the development of frank tumors, the non-malignant lesions can be caused to regress as a result of the intralesional administration of a BCG vaccine and, if so, to inquire on the effect of age on such regression.

Approach: Perform a controlled study to determine the influence of age on carcinogenesis by DMBA and of the influence of age of exposure to carcinogens upon response to immunoprophylaxis by intralesional administration of a BCG vaccine into premalignant lesions.

Progress: New contract.

Project Officers: Dr. Herbert Rapp and Ms. Judith Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: BCG Immunotherapy of Recurrent Superficial Bladder Cancer

Principal Investigator: Dr. Donald L. Lamm
Performing Organization: University of Texas
City and State: San Antonio, TX

Contract Number: NO1-CB-74192
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: To evaluate the therapeutic efficacy of intravesical and percutaneous BCG in patients with recurrent superficial bladder cancer.

Approach: A controlled randomized prospective study will be undertaken in which the administration of BCG shall be identical to the methods published in Journal of Urology 116:180-183, 1976. The study will assess the effect of BCG therapy on the rate of tumor recurrence.

Progress: A total of 43 patients have been enrolled, 22 of whom were randomly assigned to the BCG treatment group and 21 to control. Eighteen BCG-treated patients and 19 controls have been followed for a period of 3-12 months. Eight of 19 control patients have had recurrent tumors in the follow-up period, whereas 3 of 18 of the BCG-treated patients have had recurrence of bladder tumor.

Side effects of BCG therapy have been minimal: 39% have had mild gross hematuria, 95% have had dysuria which typically lasts for 1 to 2 days after each treatment, 83% have had urinary frequency, 22% have had fever (each less than 100°F), and 22% have had nausea or vomiting.

Significance to Cancer Research: A pilot study has suggested that intravesical and percutaneous BCG decreases the number of recurrences in patients with superficial bladder carcinoma. It is important to establish that this is the case with a controlled, prospective randomized trial.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $53,124

CONTRACT RESEARCH SUMMARY

Title: Intralesional Immunotherapy Prior to Surgery in the Treatment of Canine Breast Carcinoma

Principal Investigator: Dr. Steven Harris
Performing Organization: University of Texas
City and State: San Antonio, TX

Contract Number: NO1-CB-84248
Starting Date: 9/26/78 Expiration Date: 9/29/81

Goal: To evaluate in a controlled clinical study the effect of intralesional injection of BCG crude cell walls on canine breast carcinoma.

Approach: Dogs clinically free of detectable metastatic disease shall be randomly assigned to intralesional placebo prior to surgery or intralesional immunotherapy prior to surgery. The BCG crude cell walls will be provided by the National Cancer Institute. Tumor regression, tumor recurrence, disease-free interval, and survival data from the two groups shall be compared. Selected assays of humoral and cellular immunity shall be performed.

Progress: During the first four months of this contract, personnel were recruited and trained, and data collection, processing, and management systems were established. The patient referral and monitoring systems were field-tested and work on in vitro assays begun. A satisfactory dosage and schedule of administration of BCG cell wall material have been established, and seven dogs have been entered into the clinical trial and are alive and well.

Significance to Cancer Research: In considering the optimal setting for immunotherapy in the treatment of human cancer, it is important to assess the efficacy of intralesional immunotherapy prior to surgery in spontaneous tumors of outbred animals with limited disease.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $81,920

CONTRACT RESEARCH SUMMARY

Title: Detection and Characterization of Soluble Antigen-antibody Complexes in the Circulation

Principal Investigator: Dr. Brian Andrews
Performing Organization: University of Virginia
City and State: Charlottesville, VA

Contract Number: NO1-CB-84262
Starting Date: 9/28/78 Expiration Date: 9/27/79

Goal: Determine the diagnostic significance of circulating antigen-antibody complexes in the sera of cancer patients, identify the source of the antigen, and develop a sensitive assay for its presence.

Approach: Compare the results of the Raji cell radioimmunoassay, Clq binding radioimmunoassay, and mixed cryoglobulins for measurement of serum immune complexes, and determine the utility of measuring these complexes in monitoring cancer patients under various therapies. Isolate and characterize these complexes.

Progress: This contract is completing its first six months of funding. The investigator has already accrued a number of patients and studied immune complexes in their serum: 15 with colon carcinoma, 13 with breast carcinoma, 6 with benign breast lesions, and 15 with malignant melanoma. The assays used were the Raji cell radioimmunoassay, Clq binding assay, and cryoglobulin levels in serum. The Raji cell assay generally detects low levels of immune complexes (15-50 µg equiv. AHG/ml) in patients with cancer or benign breast disease but not in healthy, age and sex-matched controls. Raji values greater than 50 appear to distinguish the patients with malignant from those with benign breast disease. Immune complexes are detected less frequently by the Clq binding assay. Elevated cryoglobulins were detected in 50% of metastatic colon carcinoma, 40% of presumed breast cancer, but also in 50% of patients with benign breast lesions. It is too early to make statements regarding levels of immune complexes in patients with presumptive surgical cure because they have not been followed long enough. Immunizations are under way to raise antisera to surface-bound immune complexes, presumably continuing tumor antigens. Also, rabbits are being immunized with cryoglobulins.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $90,841

CONTRACT RESEARCH SUMMARY

Title: Diagnostic Applications of Human Tumor-associated Antigens

Principal Investigator: Dr. E. Russell Alexander
Performing Organization: University of Washington
City and State: Seattle, WA

Contract Number: NO1-CB-64068
Starting Date: 6/1/76 Expiration Date: 5/31/79

Goal: To develop and purify an antigen and antiserum which are associated with epidermoid carcinoma of the cervix. To develop methods that use antigen and antiserum in serological diagnosis of cervical cancer.

Approach: Monospecific antiserum was prepared against the tumor-associated antigen. Affinity chromatography is used to purify antigen based on monospecific antiserum used, in turn, to detect circulatory antibody and antigen.

Progress: When pure monospecific antiserum reacts against crude tumor antigen in two-dimensional immunoelectrophoresis, a single precipitin line results. This line has been demonstrated in 31 cervical cancer tissues (absent in one) and was absent in 10 other gynecologic cancers and 9 normal gynecologic tissues (including seven specimens of normal cervical tissues). The antigen is clearly protein in nature, at least in regard to the major antigenic determinant. Monospecific antiserum was utilized in examination of 39 cervical cytologic smears by an indirect immunofluorescent antibody method (20 from cervical cancer patients and 19 from women with normal cervices). Antigen was seen clearly in both membrane and cytoplasma of cervical cancer cells in all cancer cases and no controls. Observations were repeated on 2-5 smears for patients with identical results. ^{125}I-labeled cancer antigen and monospecific rabbit antiserum have been used in a radioimmunoassay to detect circulatory antigen in 13/20 sera from cancer patients but in only 2/20 sera from control patients.

Significance to Cancer Research: The aim of this study was to develop an immunodiagnostic method for early detection of cervical cancer, and to determine the relationship of such findings with the development of abnormal cervical cytology.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Adoptive Cellular Immunotherapy of Murine Tumors

Principal Investigator: Dr. Alexander Fefer
Performing Organization: University of Washington
City and State: Seattle, WA

Contract Number: NO1-CB-84247
Starting Date: 9/30/78 Expiration Date: 9/29/80

Goal: To determine the conditions and operative mechanisms for achieving optimal therapeutic effect of adoptive cellular immunotherapy used alone or in combination with chemotherapy in the treatment of several mouse tumors.

Approach: Attempts will be made to utilize in adoptive cellular immunotherapy experiments cells immunized both in vitro and in vivo. Studies will be undertaken to characterize the effector cell(s) responsible for tumor therapy as well as cells which suppress the therapeutic effect. The role of antigens for the major histocompatibility complex in sensitization to TAA and in the generation of cells therapeutically effective against established syngeneic tumors will be explored.

Progress: Studies with the C57 tumor, EL-4(G-), suggest that it should provide the necessary specificity control for studying effector and suppressor subpopulations identified in immune spleen cells used in adoptive chemoimmunotherapy of FBL-3. Techniques for secondary in vitro sensitization of primed BALB cells to LSTRA have been established. Cells thus sensitized are effective in adoptive immunotherapy of early tumor.

Work is under way to correlate in vitro and in vivo cytotoxic activity and the nature of effector and suppressor cells induced during in vitro culture. Studies are in progress to try to detect H-2 restriction in vivo by using (BALB/c x C57BL/6)F_1 cells secondarily sensitized in vitro against advanced tumors of both parental strains.

Significance to Cancer Research: The cellular component of the immune system has been demonstrated to be of importance to the rejection of transplanted syngeneic tumor in mice. This research addresses many of the theoretical and technical problems attending the use of adoptively transferred cells as a treatment for malignancies.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $143,124

CONTRACT RESEARCH SUMMARY

Title: Cells Involved in Immune Response to Tumors

Principal Investigator: Dr. Hans Wigzell
Performing Organization: Uppsala University
City and State: Uppsala, Sweden

Contract Number: NO1-CB-64033
Starting Date: 6/28/76 Expiration Date: 6/27/79

Goal: To define the physiology and characteristics of natural killer cells and other cells in the reaction against syngeneic tumor cells.

Approach: Combination of specific fractionation procedures does allow the select purification of certain subgroups of cells. Such subgroups will be analyzed as to specific tumor immune reactivity in in vitro or in vivo systems using cells in normal or immune individuals.

Progress: "Natural killer" cells in the mouse as well as in man represent a new and interesting cell type. It is endowed with the ability to rapidly lyse, especially tumor target cells, in vitro via a contactual mechanism. It was previously shown that this cell type is a thymus-independent cell, originating in the bone marrow at a certain age of the individual and quite susceptible to radioactive strontium. Chimera work has allowed the establishment of high and low NK animals by taking advantage of the genetic regulation at the stem cell level of NK generation. A highly positive correlation could be shown to exist between the NK levels in such T-deficient chimeras and their in vivo resistance to transplantation of syngeneic lymphomas. These data suggest that natural killer cells are a most decisive cell group with regard to tumor resistance in vivo. In line with these findings are the more recent results that interferon and interferon inducers including Corynebacterium parvum seem to be major activating agents for NK cells in vivo. As such agents are among the most promising for "immune therapy" of tumors, this is in line with the above concept.

Significance to Cancer Research: Deeper understanding of relevant cell types involved in in vivo resistance to syngeneic tumor cells is necessary before a predictable manipulation of the immune reactions against tumors will be possible.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: New Methods for Isolation and Characterization of Human PBLs

Principal Investigator: Dr. Hans Wigzell
Performing Organization: Uppsala University
City and State: Uppsala, Sweden

Contract Number: NO1-CB-74129
Starting Date: 9/30/77 Expiration Date: 9/29/79

Goal: To develop new methods to isolate and characterize human peripheral blood lymphocytes.

Approach: Obtain unique markers, morphological or functional, specific for a given subgroup of mononuclear cells by use of classical and innovative cell purification techniques and other methods.

Progress: In an attempt to develop unique markers for human lymphoid subpopulations several general categories of cells have been studied. Natural killer (NK) cells in humans are shown to have receptors for Fc fragments of immunoglobulin, indicating that NK and ADCC activity may be present in the same cell population. Some but not all cells with NK activity bind Helix pomatia A agglutinin. Analysis of cells from immunodeficient patients indicate that Staph. protein A is a mitogen for human B but not human T cells when proliferation is measured in serum-free medium. T-lymphoblasts induced by MLC contain a subpopulation of cells which bind a lectin from Vicia villosa, and this subpopulation appears to include the total cytotoxic T-cell activity. Soluble factors are being used to maintain human T cells in vitro at various stages of differentiation so that they can be analyzed for specific surface markers.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $55,000

CONTRACT RESEARCH SUMMARY

Title: Immunoprophylaxis of Cancer Eye in Cattle

Principal Investigator: Dr. Stephen J. Kleinschuster
Performing Organization: Utah State University
City and State: Logan, UT

Contract Number: NO1-CB-74155
Starting Date: 9/16/77 Expiration Date: 9/15/79

Goal: The goal of this research is to develop immunologic maneuvers for interfering with the progression of solid tumors from the premalignant (benign) to the malignant state and to relate the immunologic status of experimental animals to clinical events.

Approach: Intralesional administration will be made of BCG cell wall vaccine into benign ocular lesions of cattle to determine whether such treatment will retard or prevent the normal progression of these lesions to the malignant state. Additionally, the immunologic status of animals will be monitored via PPD skin testing in an attempt to correlate immunocompetence with regression, arrest, or progressive disease.

Progress: All animals have now been purchased and entered into the study. The animals were randomly divided into four experimental groups with at least 60 head in each group. The groups are (1) intralesionally administered active BCG cell wall vaccine, (2) intralesionally administered sham (inactive) vaccine, (3) local surgery, and (4) no treatment. Tissue cultured cell lines of several benign lesions have been established. These cells and blood and serum components are now available and will be used in mechanistic studies. Preliminary results indicate that vaccine can cause regression of benign lesion in some of the animals.

Significance to Cancer Research: Studies involving the immunologic interruption of the transformation of a benign precursor lesion to one of malignancy at the earliest possible stage provide valuable contributions to the overall cancer effort. Additionally, via a program of monitoring the immunocompetence of experimental animals as related to clinical events following experimentation, relevant information can be contributed to the establishment of effective immunologic testing needed in order to evaluate therapy and provide guidelines for improved human clinical protocols.

Project Officers: Dr. Herbert Rapp and Ms. Judith Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $304,260

CONTRACT RESEARCH SUMMARY

Title: Purification of Breast Tumor-associated Antigens

Principal Investigator: Dr. Robert K. Oldham
Performing Organization: Vanderbilt University
City and State: Nashville, TN

Contract Number: NO1-CB-74162
Starting Date: 9/30/77 Expiration Date: 9/29/79

Goal: Develop an assay for new tumor-associated antigens.

Approach: Isolate and purify the TAA of MCF-7, a breast cancer-derived tissue culture line, to generate heteroantisera for development of a radioimmunoassay for that antigen. Antigen isolation and purification are to be first analyzed with respect to antigen specificity in the leukocyte migration inhibition assay.

Progress: The project is concerned with purification of breast tumor-associated antigens from MCF-7 tissue culture line with intent to develop a radioimmunoassay; the work is in its second contract year. Considerable progress has been made in the physical-chemical isolation and purification of the active MCF-7 membrane product and in the biological testing of these products. The tissue culture supernatant is active as an antigen source for LMI and is specific for breast cancer patients as opposed to normal controls. Similar results have been obtained from the KCl extract of this line. The antigen(s) in these soluble membrane preparations can be purified. The active material is in the 40-100,000 dalton range and appears to be a glycoprotein. Heteroantisera are being raised in rabbits and preliminary data indicate activity of the antisera toward material from the immunizing cell population.

Significance to Cancer Research: Isolation of TAA and the development of a radioimmunoassay for carcinoma of the breast would be a valuable diagnostic and prognostic tool.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $110,746

CONTRACT RESEARCH SUMMARY

Title: Cell-mediated Reactivity of Normal Individuals to Human Tumor-associated Antigens

Principal Investigator: Dr. Robert K. Oldham
Performing Organization: Vanderbilt University
City and State: Nashville, TN

Contract Number: NO1-CB-74166
Starting Date: 9/30/77 Expiration Date: 9/29/79

Goal: Investigate the effector cell subpopulations involved in natural cytotoxicity, the antigens to which this activity is directed, and the relationship of natural activity to the immune response directed at tumor-associated antigens.

Approach: Define the natural cytotoxic activity of the K-562 model system with normal and patient populations; characterize the effector cell; investigate the model system antigens.

Progress: This contract is completing its second year. More recent data show no clear association of levels of natural cytotoxicity with age. Levels of spontaneous natural cell-mediated cytotoxicity, against the target cell line K-562, appear to be highest at extremes of age. Human data are in contrast to the age association seen in rodents which demonstrate an absence of reactivity in early life followed by an increased activity to a plateau which decreases gradually with age. There are no marked differences in cytotoxicity associated with either sex, especially in the younger age groups. This is in contrast to previously reported results which show that males were more reactive than females, at all ages in natural cell-mediated cytotoxicity. Other data demonstrate the existence of natural cell-mediated cytotoxicity prior to any influence of the extrauterine environment. Just as in the adult populations, newborns have high and low responder populations which provide stronger evidence for the genetic basis of natural cell-mediated cytotoxicity. Work continues with the extraction of cell membrane from the K-562 target in their model system in an attempt to isolate a soluble antigen preparation which inhibits NK activity and contains the active antigens.

Significance to Cancer Research: An understanding of the mechanism and target antigen system of natural cytotoxicity is necessary to understand the biologic significance of this phenomenon and to quantitate its contribution and relationship to *in vitro* assays of tumor immunity.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $77,331

CONTRACT RESEARCH SUMMARY

Title: Evaluation of the LAI Assay for Malignant Disease

Principal Investigator: Dr. Arthur Vandenbark
Performing Organization: Veterans Administration Medical Cente
City and State: Portland, OR

Contract Number: YO1-CB-80316
Starting Date: 9/30/78 Expiration Date: 9/29/79

Goal: To objectively evaluate the utility of the leukocyte adherence inhibition (LAI) assay in the monitoring of progress of cancer patients under treatment and further improve the assay for detection of reactivity.

Approach: In a double blind study test the specificity and frequency of positive LAI reactivity to various tumor extracts in patients with breast, lung, colon, and squamous cell carcinoma, using individuals with benign diseases and normal persons as controls. Perform longitudinal studies in LAI response during course of cancer therapy.

Progress: In the initial four months of the contract, tumor and control antigen preparations have been prepared from tissue from patients with squamous cell carcinoma, colon adenocarcinoma, and breast cancer. The contractor has collected tissue from patients with lung cancer for antigen preparation. Sixty-five patients and controls have been evaluated by the LAI test with these tumor and control antigen preparations. Longitudinal studies have been started on 23 patients with squamous carcinoma, six with breast cancer, and two with colon adenocarcinoma. All tests are done in a blind fashion. A higher frequency of breast cancer patients respond to breast antigens than patients with other cancers or noncancerous controls. Reactive breast cancer patients are unresponsive to normal breast antigen. In preliminary tests of the colon antigen preparation, only cancer patients responded to the extract.

Significance to Cancer Research: The objective analyses of the leukocyte adherence inhibition assay will determine its possible utility as an immunodiagnostic test for cancer.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $77,495

CONTRACT RESEARCH SUMMARY

Title: Animal Models for Bone Marrow Transplantation

Principal Investigator: Dr. Donald Metcalf
Performing Organization: Walter and Eliza Hall Institute
City and State: Parkville, Australia

Contract Number: NO1-CB-74148
Starting Date: 9/20/77 Expiration Date: 9/19/79

Goal: Develop granulocyte-macrophage and lymphocyte progenitors from transplanted marrow cells.

Approach: Agar cultures were used to monitor precursor populations of neutrophil-macrophages, eosinophils, megakaryocytes, erythroid and B-lymphocyte populations in marrow populations prior to and following transplantation to normal or pretreated mouse recipients. These cultures were also used to monitor the production and purification of the specific regulatory factors necessary to stimulate the proliferation of these cells.

Progress: The basic aim of this contract is to define the biology and proliferative potential of multipotential hemopoietic cells. Considerable progress has been made toward this goal along several approaches. In vitro colony formation assays have been established for granulocyte-macrophages, eosinophils, megakaryocytes, and erythrocytes. Soluble factors capable of stimulating the development of these different types of colonies have been isolated and preliminarily characterized. These humoral factors appear to compose a family of molecules differing in the extent and specificity of glycosylation. Use of these factors (e.g., GM-CSF and erythropoietin) in competition assays indicates that progenitors capable of forming colonies in vitro are already committed along one differentiation pathway and are not multipotential stem cells. In vivo experiments indicate that bacterial products such as endotoxin and lipid A can increase titers of the regulating humoral factors on first exposure but result in decreased titers on repeated treatment. The use of the fluorescence activated cell sorter to sort stem cells on the basis of surface antigenicity and cell size has permitted fractionation of such cells into subpopulations which give rise to only a single type of colony contrasted to mixed populations which can give rise to all colony types.

Significance to Cancer Research: The present studies are designed to develop more effective procedures for transplantation of marrow cells in leukemic patients and in patients with solid tumors undergoing autologous marrow reinfusion following chemotherapy and/or irradiation.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Detection and Development of H-2 Recombinant and Mutant Strains

Principal Investigator: Dr. Donald C. Shreffler
Performing Organization: Washington University
City and State: St. Louis, MO

Contract Number: N01-CB-74154
Starting Date: 3/1/77 Expiration Date: 2/28/80

Goal: The detection and fixation in congenic inbred strains of new recombinant and mutant H-2 haplotypes, particularly variants involving the I (immune response) region of the H-2 gene complex.

Approach: Screen large numbers of progeny from crosses involving different H-2 haplotypes for: (1) testing of mutagens that may enhance the frequencies of recombination and mutation; (2) screening of progeny of newly arisen recombinants for second-step recombination events; and (3) development of MLR and Ia serotyping methods for detection of I region recombinants and mutants.

Progress: Over the past year some 4,400 progeny from 17 different crosses were screened and 21 putative new intra-H-2 recombinants were detected. Of those that have been proven by progeny test so far, 11 are being carried as new congenic lines and 8 are still undergoing testing. By screening 520 progeny of mutagen-treated heterozygous males, three possible recombinants have been found and are being progeny tested. Mutagen treatment seems to enhance intra-H-2 recombination, but there may be some instability in the newly recombinant chromosomes. A pilot test of MLR screening methodology has been started and among 100 heterozygous progeny screened, three have been found to significantly stimulate a presumed syngeneic test cell. Progeny tests are under way and it remains unclear whether these are true genetic variants. An improved method of screening for I region variants may permit efficient detection of both recombinants and mutations among the same progeny.

Significance to Cancer Research: The H-2 complex plays a critical role in susceptibility to oncogenic viruses. The new strains produced will aid in the definition of the genes that control susceptibility. The products of K, D, and I regions of the complex are involved in cell-mediated reactions against neoplastic cells. These new strains will provide material for characterization of these products.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $83,455

CONTRACT RESEARCH SUMMARY

Title: Antibody-Drug Conjugates in Immunotherapy

Principal Investigator: Prof. Ruth Arnon
Performing Organization: Weizmann Institute
City and State: Rehovot, Israel

Contract Number: NO1-CB-64049
Starting Date: 6/30/76 Expiration Date: 6/29/79

Goal: To explore the feasibility of an immunochemotherapeutic approach--the use of antitumor antibodies as specific carriers of anti-cancer drugs for local delivery of the drugs to the tumor site.

Approach: Anti-cancer drugs such as daunomycin and adriamycin are attached covalently either to the IgG fraction of antitumor sera or to purified antitumor antibodies. The cytotoxic effect of the resulting conjugates on the tumor cells is tested both in vitro and in vivo, and compared to the effect of free drug, free antibodies, their mixtures, or conjugates of the drugs with normal immunoglobulins, and its mechanism of action is investigated.

Progress: Studies of the mechanism of action of the daunomycin conjugates were performed, and it appears that the drug conjugate is taken up by the cells in bound form and that the free drug is released by acid hydrolysis to result in cell death. Efforts were made to develop specific antitumor antibodies in several mouse and rat tumor systems. The tumor systems used were the A/J lymphoma system, the Lewis lung tumor, and a rat chemically induced colon carcinoma. Studies exploring the "homing" ability of iodinated anti-YAC antibodies were continued. A specific accumulation of antibodies to various tumor-infested tissue was observed in mice at late stages of tumor development. This was not observed in the early stages of tumor development or when tumor was implanted subcutaneously rather than intraperitoneally.

Significance to Cancer Research: The suggested approach is a combination of chemotherapy and immunotherapy in an attempt to localize the cytotoxic drug in the tumor site while maintaining low systemic concentration.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Molecular Studies of Specific Cell-mediated Cytotoxicity

Principal Investigator: Dr. Gideon Berke
Performing Organization: Weizmann Institute
City and State: Rehovot, Israel

Contract Number: NO1-CB-74183
Starting Date: 9/30/77 Expiration Date: 9/29/79

Goal: Elucidation of the mechanism whereby cytotoxic T lymphocytes (CTL) interact with and kill target tumor cells (TC) in vitro.

Approach: Isolate and characterize a cell-free, functionally reactive component of CTL and study its mode of action on the target cells; determine the role of membrane lipid composition and microviscosity on CTL-TC interaction; do biochemical studies on CTL-TC contact regions; evaluate the possibility that TC has an active role in the lytic process.

Progress: The workscope of this contract is aimed at an understanding of the mechanism by which cytotoxic T lymphocytes (CTL) lyse allogeneic and syngeneic target cells (TC). During the period covered in this progress report, the investigators have demonstrated that enucleated cytoplasts which contain most normal cytoplasmic components of the intact lymphocyte can specifically bind appropriate targets. No data on the lytic potential of these cytoplasts are given. Additional studies have indicated that a cell-surface component of CTL previously identified as immunoglobulin light chain has no relationship to immunoglobulin. The most interesting experiments reported are those in which heat treatment of CTL or exposure to low concentrations of formaldehyde can be used to remove lytic activity while leaving binding of T cells and targets intact. The morphological observations are also of interest.

Significance to Cancer Research: The precise understanding of the interaction of lymphocytes and tumor cells could (1) clarify the role of the immune system in the response against tumors, (2) provide reason(s) for its failure in the tumor-bearing patients, and (3) contribute to a more rational approach to immunotherapy of tumors.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $112,605

CONTRACT RESEARCH SUMMARY

Title: Induction of Functional Differentiation of T cells

Principal Investigator: Dr. Martin Haas
Performing Organization: Weizmann Institute
City and State: Rehovot, Israel

Contract Number: NO1-CB-74180
Starting Date: 9/30/77 Expiration Date: 9/29/79

Goal: To establish methods for in vitro leukemogenesis of lymphocytes on thymus reticuloepithelial cells through analysis of the role of retroviruses, identification of target lymphocytes, and purification of different types of thymus reticuloepithelial cells.

Approach: Establish culture conditions for growing reticuloepithelial cell monolayers from leukemic and normal mice. The monolayer cultures will be analyzed for cell type, retrovirus production, and sensitivity to infection. The reticuloepithelial cell monolayers will be used in cocultivation experiments with lymphocytes in order to study differentiation of different types of lymphocytes and their transformation into leukemic cells.

Progress: The contract is completing its second year. The type and potency of the virus isolates are the major factor in initiation of complete in vitro lymphomagenesis. With this in mind, the contractor has sought to propagate, clone, and characterize new virus isolates from thymic and non-thymic lymphomata of X-ray-induced C57BL/6 mice. Dualtropic viruses were isolated from adherent, stromal cell lines grown from hematopoietic organs of reticulum cell neoplasm (RCN)-bearing mice. Some of these viruses induced RCN in vivo with high efficiency and short latent period. The RCN-inducing dualtropic isolates were interfered with by eco- and xenotropic viruses and could be neutralized with antisera against them. Thus, these dualtropic isolates are probably (gp70) envelope recombinants. There was no correlation between in vitro induction of cytopathic effect in mink cells and in vivo induction of RCN. The RCN and thymic lymphoma (TL) virus isolates of X-ray-induced C57BL/6 mice are different viruses capable of simultaneous differential induction of the two diseases in the same mouse. The contractor was able to perform in vitro lymphomagenesis experiments using the high-titer, potent lymphoma-inducing viruses he now has.

Significance to Cancer Research: In vitro induction of lymphosarcomas, isolation, and analysis of the causative agents could lead to an understanding of the induction of spontaneous lymphosarcomas.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $99,000

CONTRACT RESEARCH SUMMARY

Title: Genetic Control of Susceptibility to Tumors

Principal Investigator: Dr. Nechama Haran-Ghera
Performing Organization: Weizmann Institute
City and State: Rehovot, Israel

Contract Number: NO1-CB-74151
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: To study genetic control of susceptibility to tumors.

Approach: Investigate the number, genetic map position, mutual relationship, and function of H-2 linked resistance genes to murine leukemia, including a study of genetic regulation of the phases in leukemogenesis.

Progress: This study is an investigation of the genetic control of immune responses against leukemia induced by variants of the Rad LV viral complex. It appears that leukemogenesis with A-Rad LV, D-Rad LV, and Duplan's Rad LV has H-2 linked components other than Fv-1. The viruses studied fall into two different categories. One group (A-Rad LV and Duplan's Rad LV) is oncogenic only in animals of the same H-2 haplotype as that of mice from which the virus was originally isolated while the other (D-Rad LV and Rad LV old) is oncogenic only in strains allogeneic to the H-2 haplotype from which the virus was isolated. Practically all H-2 loci (including those of the Ir region) are involved in resistance to these viruses, indicating an influence of different immune responses. Preleukemic cells could be induced in nu/nu mice, indicating the thymic independence of the stage of oncogenesis. Peanut lectin was shown to preferentially induce aggregation of these preleukemic cells.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Immunobiology of Metastases

Principal Investigator: Dr. Shraga Segal
Performing Organization: Weizmann Institute
City and State: Rehovot, Israel

Contract Number: NO1-CB-74185
Starting Date: 9/30/77 Expiration Date: 9/29/79

Goal: Further analysis of the antigenic diversity of lung metastases generated by the 3LL tumor.

Approach: Generation of cytotoxic T-lymphocytes in culture against M-3LL and in L-3LL and cross-testing their cytotoxic effects.

Progress: This contract is completing its second year. The contractor has made progress regarding differences between M-3LL (metastatic) and L-3LL (primary) cells. The M-3LL cells have a higher resistance to the effector function of NK cells than L-3LL cells do. It appears that NK cells, in addition to other specific immune effector systems, may participate as a selective force in processes leading to diversification and generation of deviant malignant tumor cell clones, which may disseminate and develop distant metastases in spite of the function of specific and nonspecific antimetastatic effector mechanisms. The metastatic cell membranes are more fluid than local tumor cell membranes. Surgical excision of local tumor leads to enhanced development and progression of pulmonary metastases. This metastatic acceleration could be abrogated by reinoculating the operated animal with live homologous tumor cells. The abrogation of metastatic progression by live tumor cells is dose dependent. Other experiments indicate that the spleen is involved in the balance between local and metastatic populations. The postsurgical development of metastases and survival time following primary tumor excision were found to be dependent on the rate of progression of the primary tumor.

Significance to Cancer Research: The fact that metastases differ antigenically from their primary tumor ancestry may contribute to our understanding of mechanisms by which metastases avoid immune destruction by the host. In addition, these findings may lead to more rational approaches to the problem of immunotherapy and control of metastasis spread and growth.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $56,100

CONTRACT RESEARCH SUMMARY

Title: Isolation and Characterization of Human PBLs

Principal Investigator: Dr. Nathan Sharon
Performing Organization: Weizmann Institute
City and State: Rehovot, Israel

Contract Number: NO1-CB-74163
Starting Date: 9/30/77 Expiration Date: 9/29/79

Goal: Develop new methods for isolating and characterizing human peripheral blood lymphocytes.

Approach: Develop procedures for cell fractionation by selective agglutination with lectins. Mononuclear cells from both healthy donors and cancer patients will be screened for their ability to bind fluorescent derivatives of peanut, soybean and wheat germ agglutinins.

Progress: This contract is completing its second year. Recent work has focused on using human thymocytes since lectin-binding techniques were worked out in the mouse system. The majority (60-80%) of human thymocytes bind to peanut agglutinin (PNA). Upon removal of sialic acid with neuraminidase all cells bound to the lectin. PNA^+ cells did not respond to the mitogenic stimulation of PHA nor did they respond in the mixed lymphocyte test. PNA^- cells respond to both stimuli. Therefore, it seems that human PNA^+ thymocytes, like the mouse system, are functionally immature. PNA provides a unique tool for separating mature from immature thymocytes. PNA^+ cells bear the receptor for E-rosette formation, suggesting that the masking of the PNA receptor by sialic acid occurs at a later stage of T-cell maturation than the expression of the E-rosette receptor; therefore, PNA is a more sensitive marker of T-cell immaturity than E-rosetting. In studies on peripheral blood mononuclear cells of normal and leukemic donors, less than 2% of the normal PBLs bound PNA. The pathologic type of leukemia determined binding for those donors: greater than 50% of the PBLs bind in ALL, stem cell leukemia, and myeloid leukemia, while CLL patients' cells were PNA^-. However, in three CLL patients a subpopulation of PNA^+ lymphocytes was found. Studies continue on using soybean agglutinin (SBA) to separate T and B cells. These studies include surface marker and immunobiological identification. Human cord blood lymphocytes have a PNA^+ fraction which responds poorly to PHA and Con A as offered to the PNA^- fraction.

Significance to Cancer Research: The presence of PNA receptors on the blastic cells of patients with acute leukemia could be of therapeutic importance, since they may serve as selective targets for PNA-bound drugs.

Project Officer: Ms. Judith M. Whalen
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $93,300

CONTRACT RESEARCH SUMMARY

Title: Altered Membrane Microviscosity: Effects on Tumor Cell Immunogenicity

Principal Investigator: Dr. Meir Shinitzky
Performing Organization: Weizmann Institute
City and State: Rehovot, Israel

Contract Number: NO1-CB-74193
Starting Date: 9/30/77 Expiration Date: 3/31/79

Goal: To increase the antigenicity of murine tumor cells by altering membrane microviscosity.

Approach: Tumor cells from several mouse tumors will be treated with liposomes of varying cholesterol/phospholipid ratios on fatty acids and their derivatives in attempts to modify membrane composition. Correlation between microviscosity and the degree of exposure of various membrane antigens will be studied. In vitro studies will focus on the effect of lipid treatment of tumor cells on the sensitization of spleen cells. In vivo work will evaluate the degree of immunity induced by prophylactic injection of altered tumor cells, and ultimately the use of altered tumor cells in therapeutic modes.

Progress: Modulation of the expression of well-defined antigens by changing the lipid microviscosity has been observed with the common blood group antigens and the θ antigen of T cells. Passive modulation of weaker antigens is now being studied. In parallel, in vivo and in vitro screenings of the antigenicity of a series of tumor cell lines have been undertaken. The effects of increased and decreased membrane microviscosity on the immunogenicity of syngeneic tumors have been studied. Treated cells were irradiated and inoculated i.p. 10^7 cells per mouse in separate groups. In control experiments mice were inoculated with irradiated untreated cells. Following three immunizations, mice were challenged with viable tumor cells and were observed for tumor growth and survival. In virtually all cases, the observed immunization in the groups immunized with cells of increased membrane microviscosity was greater than in the untreated animals or in animals treated with cells which were only irradiated.

Significance to Cancer Research: The increase by passive modulation in immunogenicity of syngeneic tumors may be used as a clinical immunotherapy tool.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Intratumoral BCG Immunotherapy Prior to Surgery for Carcinoma of the Lung

Principal Investigator: Dr. Richard Mattnay
Performing Organization: Yale University
City and State: New Haven, CT

Contract Number: N01-CB-74191
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: To evaluate the therapeutic efficacy of intratumoral BCG prior to surgery for carcinoma of the lung.

Approach: Patients with potentially resectable carcinoma of the lung will be randomly assigned to treatment with intratumoral BCG (administered by transthoracic needle or fiberoptic bronchoscope) two weeks prior to surgery or surgery alone. Survival and disease-free interval will be analyzed in the two treatment groups.

Progress: Of the 34 patients entered on study, 27 are alive and well without recurrence. There have been seven recurrences and/or deaths: three among Stage III BCG-injected patients, two in Stage III control patients, and one each in a Stage I BCG and a Stage I control patient, respectively.

Patients who received intratumoral BCG uniformly developed fever within 24 hours of injection (average: 102.5°F). In the resected specimens of BCG-treated patients, a lymphocytic, granulomatous peritumoral infiltrate was noted histologically in 80% of the cases. Acid-fast bacilli also were seen on smear and/or grown from sputum, tumor, or regional lymph nodes.

Significance to Cancer Research: Relatively little is known about the value of immunotherapy prior to surgery in early clinically nondisseminated human malignancies. It is important to assess this therapeutic approach in a controlled, randomized prospective study.

Project Officer: Harriet L. G. Gordon, M.D.
Program: Immunology Section
Technical Review Group: Tumor Immunology Committee
FY 79 Funds: $74,400

CONTRACT RESEARCH SUMMARY

Title: Maintain an Animal Holding Facility and Provide Attendant Research Services

Principal Investigator: Dr. Diane Hernandez
Performing Organization: Cor Bel Laboratories, Inc.
City and State: Rockville, MD

Contract Number: NO1-CB-64024
Starting Date: 11/1/75 Expiration Date: 10/31/79

Goal: Maintain colonies of inbred mice (7,500 animals), inbred rats (800 animals), and rabbits (25 animals) and carry out selected breeding protocols with these animals as specified by the project officer. These animals are to be maintained in support of intramural research programs in the Immunology Branch, NCI.

Approach: Colonies of mice, rats, and rabbits are to be housed and fed according to National Research Council standards. Technical manipulations and breeding are to be carried out as directed by the project officer.

Progress: Performance on this contract has been highly satisfactory. The animal colonies have been established and are being maintained according to National Research Council standards. Animal health has, in general, been excellent, and breeding protocols have been satisfactory. Recordkeeping and transferring of animals to and from the NIH Campus have all been satisfactory.

Significance to Cancer Research: This animal colony is necessary in support of intramural research programs in the Immunology Branch of NCI. Many of these programs are concerned with the immune response to cancer.

Project Officer: Dr. David H. Sachs
Program: Tumor Immunology Program
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 79 Funds: $258,475

CONTRACT RESEARCH SUMMARY

Title: NCI Histocompatibility Typing Center

Principal Investigator: Dr. A. H. Johnson
Performing Organization: Duke University
City and State: Durham, NC

Contract Number: NO1-CB-53890
Starting Date: 4/1/78 Expiration Date: 3/31/80

Goal: To study HL-A, A, B, C, DR, cell-surface antigens on patients and in normal individuals to attempt to establish a correlation between a particular antigen and a disease condition. Families in which more than one member has a particular disease will be studied to determine if the major histocompatibility complex contributes to the disease state.

Approach: Complement dependent cytotoxicity studies will be performed with cell and serum provided by the project officer.

Progress: HLA, A, B, C, DR typings were performed on a panel of normal individuals and individuals with the following disease diagnoses: diabetes mellitus with and without insulin allergy, systemic lupus erythematosus, Sicca syndrome, acute lymphocytic leukemia, Hodgkin's disease, and malignant melanoma. The significant findings are as follows: Patients with the Sicca syndrome demonstrated an increased frequency of the DRW-3 antigen compared to the normal population. In comparing this disease population to patients with Sicca syndrome with rheumatoid arthritis, the DRW 3 antigen was replaced by DRW 4. Thus, it appears that the genes of the major histocompatibility complex influence the manifestation of the disease syndrome. Patients with systemic lupus erythematosus were found to have elevated DRW-2, DRW-3 antigens compared to the normal population and, in addition, one antisera IA 715 reacted with 75% of the patients compared to 14% of the control population. This disease appears to be of autoimmune etiology. The antigen detected by the antisera IA 715 may mark immune response genes. Analysis of data generated in typing families in which disease association has been determined has resulted in important conclusions related to the genetics of the antigens in the DR region of the major histocompatibility complex. Studies performed thus far indicate that several gene loci control the expression of these antigens, a finding which contributes to the knowledge of the genetics of the MHC.

Significance to Cancer Research: Establishing correlations of antigens controlled by genes of the major histocompatibility complex has suggested a fundamental biological role for these genes in disease manifestations. This information establishes the concept of immune response genes in man. As cancer is a complex biologic entity, the investigation and identification of immune response genes potentially adds important information in our understanding of the lack of immune response to neoplastic transformation.

Project Officer: Dean L. Mann, M.D.
Program: Tumor Immunology Program
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
Fy 79 Funds: $257,453 ($224,612 provided by the NCI Immunology Branch, $12,841 provided by NIAID, and $20,000 provided by NCI Epidemiology Branch)

CONTRACT RESEARCH SUMMARY

Title: Systems Analysis and Informational Service in Registries of Clinical Protocols

Principal Investigator: Ms. Molly A. Wolfe
Performing Organization: Informatics, Inc.
City and State: Rockville, MD

Contract Number: NO1-CB-53883
Starting Date: 5/2/75 Expiration Date: 11/1/79

Goal: To provide analyzed integrated information regarding ongoing clinical immunotherapy protocols to cooperating clinicians.

Approach: Standardized data accumulation and storage methods have been developed for characterizing clinical trials on immunotherapy. Protocols submitted to the Registry are analyzed, indexed, and periodically updated; an annual Compendium of these protocols is prepared and distributed to participating clinical investigators. In addition, an address file of approximately 3,000 physicians is maintained and periodically updated. Periodic reports describing current Registry activities and related topics in immunotherapy are published and distributed to those on the general mailing list.

Progress: The sixth edition of the Compendium on Tumor Immunotherapy Protocols was published in September 1978. Prior to its publication, considerable effort was spent in obtaining accurate update information on the 430 protocols contained in the Registry, and in the analysis of 63 new protocols. The Compendium includes a summary of each protocol in the Immunotherapy Registry and is indexed by investigator, tumor, immunotherapy agent, and geographic location. A mini compendium, containing an indexed listing of protocol titles and investigators, was also prepared and distributed to all the physicians on the Registry mailing list.

Significance to Cancer Research: The International Registry of Tumor Immunotherapy is a valuable source of ongoing clinical trials in immunotherapy. The annual Compendium of Tumor Immunotherapy is a mechanism for a rapid exchange of current information among clinicians engaged in immunotherapy, and further, it may encourage and aid in the design of new protocols.

Project Officer: Mrs. Susan Ficker
Program: Managed by the Tumor Immunology Program in Immunotherapy
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 79 Funds: $43,935 from ICRDB, OD/NCI

CONTRACT RESEARCH SUMMARY

Title: Induction, Transplantation, and Preservation of Plasma Cell Tumors in Mice

Principal Investigator: Dr. Jean Dealy
Performing Organization: Litton Bionetics, Inc.
City and State: Bethesda, MD

Contract Number: NO1-CB-92142
Starting Date: 6/23/69 Expiration Date: 2/28/79

Goal: Transplantation, preservation, induction, and shipping of plasmacytomas, T- and B-cell lymphomas in mice, and breeding of rare strains of mice, hybrids, and wild mice.

Approach: To maintain a frozen tumor bank of about 1,100 tumor lines of plasmacytomas, B- and T-cell tumors, which is the source of reference tumors for both intramural NCI and outside investigators. To supply tumor lines, tumor products (ascites, serum, urine, purified myeloma proteins), and antisera to NCI for ongoing research. To maintain a special screening bank of 250 typed myeloma proteins derived from plasmacytomas. These proteins are classified for V-isotypes and antigen binding activities. To breed conventional mice for production of various congenic strains and mice used in plasmacytomagenesis experiments, and to maintain these mice during plasmacytomagenesis. To maintain a colony of rare species and subspecies of Mus.

Progress: Contractor has improved its expertise in developing and testing Ig-V-region isotypic and idiotypic antisera for classifying V-regions of the 250 myeloma proteins in the special screening bank. The QUPC52 1,α6 DEX idiotype has been characterized and found to be a new immune response marker. Plasmacytoma induction experiments are in full operation. The role of age on susceptibility to plasmacytomagenesis is in progress. Twenty new congenic BALB/c strains to be used in plasmacytomagenesis experiments are being developed using genes from resistant strains DBA/2 and C3H/HeJ. Strains are in 5th backcross. Contractor continues production of Mus cervicolor, caroli, shortridgei, pahari, poschiavinus, hispanicus, and Mus m. castaneus, musculus and molossinus and 6 to 10 European and North African musculus subspecies. LBI made 1,450 shipments of mice tumors and products to other investigators. Contract has supported ongoing immunochemical-structural studies in NCI, NIAID, and NIAMD.

Significance to Cancer Research: Supplies essential biological material for investigators studying the biology of neoplastic plasma cells, tumor immunology, the genetics of immunoglobulins, and immunoglobulin synthesis.

Project Officers: Dr. Michael Potter and Dr. David H. Sachs
Program: Tumor Immunology Program
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 79 Funds: $33,146

CONTRACT RESEARCH SUMMARY

Title: Induction, Transplantation, and Preservation of Plasma Cell Tumors in Mice and the Maintenance of Special Strains

Principal Investigator: Dr. Jean Dealy
Performing Organization: Litton Bionetics, Inc.
City and State: Bethesda, MD

Contract Number: NO1-CB-94326
Starting Date: 3/1/79 Expiration Date: 1/31/82

Goal: Transplantation, preservation induction, and shipping of plasmacytomas, T- and B-cell lymphomas in mice. Breeding of rare strains of mice, hybrids, and wild mice.

Approach: To maintain a closed conventional colony of BALB/c, CB-20, CAL-20 mice, and other BALB/c sublines for use in plasmacytoma induction and for the maintenance of a bank of transplantable plasmacytomas and other lymphocytic tumors. The mice in this environment are suitable for long-term plasmacytoma induction studies and for the generation of congenic strains of the BALB/c background. New BALB/c congenic lines, carrying genes from the plasmacytoma-genesis-resistant DBA/2 background, are being bred for use in plasmacytoma induction studies to identify genes involved with the genetic susceptibility of plasmacytomagenesis. In addition, to maintain a screening bank of myeloma proteins for the detection of new antigen binding myeloma and to maintain a wild mouse colony.

Progress: The contractor has maintained 1,500 tumor lines including 238 new tumors,and has made 1,822 shipments to intramural and outside investigators. Approximately 10 different BALB/c congenics carrying DBA/2 markers are at N6 and are being treated for initial plasmatomagenesis tests. The BALB/JAX sub-line has proven to be plasmacytoma resistant on first test. Studies on the effect of age, sex, and dose of pristane are nearing completion. New rapid, more efficient induction methods have been developed. The wild mouse colony is producing sufficient numbers of Mus caroli, cervicolor, pahari, spretus, cookii, and Mus musculus subspecies for continuing studies on the genetics of immunoglobulins. Continued studies of the biological activity of myeloma proteins have produced new antigen binding myeloma proteins form different allotype backgrounds.

Significance to Cancer Research: Provides essential support for the study of plasmacytomagenesis (carcinogenesis) with the specific goal of determining the genetic basis of susceptibility to tumor induction by mineral oil. Supplies essential biological material for investigators studying the biology of neoplastic plasma cells, tumor immunology, the genetics of immunoglobulins, and immunoglobulin synthesis.

Project Officer: Dr. Michael Potter
Program: Tumor Immunology Program
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 79 Funds: $364,004

CONTRACT RESEARCH SUMMARY

Title: Maintenance and Development of Inbred and Congenic Resistant Mouse Strains

Principal Investigator: Dr. Jean Dealy
Performing Organization: Litton Bionetics, Inc.
City and State: Kensington, MD

Contract Number: NO1-CB-94325
Starting Date: 2/1/79 Expiration Date: 1/31/82

Goal: To maintain a colony of inbred pedigreed strains of mice which are needed to support ongoing NCI intramural research in transplantation immunology.

Approach: The contractor maintains a colony of approximately 40 special inbred and congenic resistant strains of mice by pedigreed brother-sister mating. Quality control testing is carried out at each generation by cytotoxicity typing of animals from each strain. Alloantisera are raised between mouse strains to assist in this quality control typing and sera and animals are shipped by the contractor to collaborating investigators at NIH and elsewhere.

Progress: The contractor has maintained all inbred and congenic resistant strains of mice in excellent condition. Breeding of each strain and of hybrid strains, record keeping, and quality control testing have all been highly satisfactory. A backcrossing program has been instituted for all congenic resistant strains in order to keep the backgrounds of these strains identical. This involves backcrossing of each congenic to the reference background line once every 6-10 generations. This program has also been very satisfactory to date. Two new recombinant H-2 haplotyes have been identified during the process of this backcrossing, and these have been bred to homozygosity and established as two new valuable inbred congenic strains.

Antisera for histocompatibility antigen typing have been prepared in a variety of combinations and have been found to be excellent reagents. A series of new strain-restricted typing sera have been produced in order to identify each strain in the colony and distinguish it from all other strains. Shipping of animals and sera to collaborating investigators at NIH and elsewhere has been very satisfactory. The animals shipped from these pedigreed colonies have generally been of excellent health and have provided breeding stock for the production of larger numbers of experimental animals in numerous laboratories.

Significance to Cancer Research: This animal facility is needed for the breeding and maintenance of these inbred congenic resistant strains of mice. These animals make possible research on individual histocompatibility antigens and, in particular, the role of the major histocompatibility complex in the transplantation of tissues and cells and in the immune response to cancer.

Project Officer: Dr. David H. Sachs
Program: Tumor Immunology Program
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 79 Funds: $205,340

CONTRACT RESEARCH SUMMARY

Title: Measurement of Immunological Reactivity to Human Cancer

Principal Investigator: Dr. Jack Dean
Performing Organization: Litton Bionetics, Inc.
City and State: Kensington, MD

Contract Number: NO1-CB-63975
Starting Date: 9/1/78 Expiration Date: 4/30/80

Goal: To study the immune responses of cancer patients to tumor-associated antigens and to correlate results of different assays with each other and with clinical status of patients. To provide support for the Laboratory of Immunodiagnosis, NCI, for its detailed studies of cell-mediated immunity to human cancer.

Approach: Immunological tests to evaluate and monitor the immunocompetence of patients and their response to tumor-associated antigens include lymphocyte stimulation by mitogens, recall antigens, and autologous tumor antigens; rosette assays for enumeration of T cells and their subpopulations; leukocyte migration inhibition assays against recall antigens and tumor-associated antigens; and studies of direct cytotoxicity and antibody-dependent cell-mediated cytotoxicity against various human tumor cell lines.

Progress: Since a considerable number of patients with carcinoma of the lung or breast have been studied on this project over the past several years, it was important to determine whether the test results correlated with clinical course of disease. In lung cancer, depressed lymphoproliferative responses in mixed leukocyte cultures performed at about one month after surgery in stage I patients correlated with a high rate of development of recurrent disease. In breast cancer, a significantly higher rate of recurrence was associated with low or negative lymphoproliferative responses to autologous tumor antigens, with mixed leukocyte culture responses in the normal range, and with positive reactivity in the leukocyte migration inhibition assay after surgery. These results indicate that some of the immunologic tests performed as a part of this project may be useful in substaging patients with lung and breast cancer. Another emphasis in the project has been on the detailed monitoring of patients receiving immunotherapy with BCG or C. parvum. It was found that after one inoculation with either agent, there was a rapid increase in cytostatic activity of monocytes and natural killer cells and a concomitant depression in lymphoproliferative responses to PHA or in mixed leukocyte cultures. These studies appear to be promising in providing new information on the immunologic effects of immunotherapeutic manipulation.

Significance to Cancer Research: These studies are providing important information regarding the usefulness of various assays of cell-mediated immunity for immunodiagnosis and for monitoring the clinical course of cancer patients. In addition, important new information on the mechanisms underlying cellular immunity in cancer is being obtained.

Project Officer: Dr. Ronald B. Herberman
Program: Tumor Immunology Program
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 79 Funds: $293,609

CONTRACT RESEARCH SUMMARY

Title: Biologic Studies of Solubilized Tumor Antigens

Principal Investigator: Dr. Jack Dean
Performing Organization: Litton Bionetics, Inc.
City and State: Kensington, MD

Contract Number: NO1-CB-63987
Starting Date: 9/29/76 Expiration Date: 9/28/79

Goal: To develop a rapid, reproducible, and specific assay to monitor solubilization and purification of various TSTAs. Assays must have some relationship to tumor rejection activity. To study cell-mediated mechanisms using soluble antigens to immunize syngeneic mice.

Approach: The biologic activities of detergent-solubilized and of various chromatographic fractions of plasma membranes bearing TSTA (tumor rejection activity) are studied by several methods: lymphocyte stimulation, inhibition of macrophage migration, Winn assay, and delayed hypersensitivity reactivity (in the foot pad). At the same time tumor rejection activity of these same materials is being assayed at LCB. TSTA activities of chemically induced and of DNA and RNA virus-induced syngeneic neoplasms of the mouse are being studied.

Progress: Detergent-solubilized (NP40 and deoxycholate) and partially purified fractions of TSTA following chromatography on agarose-polyacrylamide columns have shown specific activity in the agarose droplet assay for inhibition of macrophage activity and in the foot pad assay for delayed hypersensitivity reactivity. Nanogram amounts (protein) have shown activity in those fractions known to have tumor rejection activity in the syngeneic mouse. Fractions obtained from Meth-A, a chemically induced sarcoma, and from mKSA, an SV40-induced sarcoma prepared by LCB, were specifically active whereas no specificity could be obtained in these assays using materials prepared in the same way from leukemic cells induced by RNA viruses; these latter neoplasms are continuously releasing infectious virus and this may be a complication. Although specificity appeared to be attained, the several assays did not lend themselves to a quantitative assay and thus did not appear useful as assays in the LCB purification procedures. Further, a precise relationship of these assays to tumor rejection has not been established.

Significance to Cancer Research: Knowledge gained through a study of the biology and chemistry of specific tumor antigens on the cell surface of neoplastic cells, transformed by RNA and DNA viruses and by carcinogenic chemicals, will hopefully lead to an understanding of the mechanisms of cancer induction and maintenance of the neoplastic state and in the prevention and control of neoplasms in man.

Project Officer: Dr. Lloyd W. Law
Program: Tumor Immunology Program
Technical Review Group: Immunology Support (Ad Hoc Review)
FY 79 Funds: $266,627

CONTRACT RESEARCH SUMMARY

Title: Transplantation, Induction, and Preservation of Plasma Cell Tumors in Mice and the Maintenance of Special Strains

Principal Investigators: Ms. Martha McGowan and Dr. Chris Mannis
Performing Organization: Litton Bionetics, Inc.
City and State: Bethesda, MD

Contract Number: NO1-CB-94326
Starting Date: 3/1/79 Expiration Date: 1/31/82

Goal: Transplantation, preservation, induction, and shipping of plasmacytomas, T- and B-cell lymphomas in mice, and breeding of rare strains of mice, hybrids, and wild mice.

Approach: To maintain a frozen tumor bank of about 1,100 tumor lines of plasmacytomas, B- and T-cell tumors, which is the source of reference tumors for both intramural NCI and outside investigators. To supply tumor lines, tumor products (ascites, serum, urine, purified myeloma proteins), and antisera to NCI for ongoing research. To maintain a special screening bank of 250 typed myeloma proteins derived from plasmacytomas. These proteins are classified for V-isotypes and antigen binding activities. To breed conventional mice for production of various congenic strains and mice used in plasmacytomagenesis experiments, and to maintain these mice during plasmacytomagenesis. To maintain a colony of rare species and subspecies of Mus.

Progress: The closed conventional colony of BALB/c mice has proved invaluable for performing plasmacytoma induction experiments as minimal losses due to nonspecific causes are sustained during the required period of observation. Induction studies testing the effects of age, sex, and dose of pristane are nearing completion. New BALB/c congenic strains carrying DBA/2 loci are being bred for tests to determine presence of genes conferring resistance to peritoneal plasmacytomagenesis. These include congenic carrying markers from chromosomes 1, 4, 5 and 2 other undesignated chromosomes. BALB/JAX bred at LBI is currently being tested to confirm resistance of this strain. The wild mouse colony has acquired new European and North African species. New antigen binding myeloma proteins have been discovered including a phosphocholine binding protein from CB-22. Myeloma proteins have been tested for their ability to bind other myeloma proteins (4,000 to 5,000 tests) without discovery of a clear reactor. Contractor has supplied the usual large number of tumors and tumor products to ongoing research and collaborators.

Significance to Cancer Research: Supplies essential biological material for investigators studying the biology of neoplastic plasma cells, tumor immunology, the genetics of immunoglobulins, and immunoglobulin synthesis.

Project Officer: Dr. Michael Potter
Program: Tumor Immunology Program
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 79 Funds: $364,004

CONTRACT RESEARCH SUMMARY

Title: Melanoma Cell Vaccine in In Vitro Assays for Humoral and Cellular Cytotoxicity

Principal Investigator: Dr. Edwin Matthews
Performing Organization: Litton Bionetics, Inc.
City and State: Kensington, MD

Contract Number: NO1-CB-53916
Starting Date: 5/9/75 Expiration Date: 5/10/80

Goal: To prepare cultured melanoma cells for immunotherapy vaccine and carry out in vitro cytotoxicity tests to measure immune responses of patients to the vaccine.

Approach: (1) Grow human melanoma cells free of contaminants, treat cells with neuraminidase, freeze under sterile conditions, and provide cells as needed for human immunotherapy. (2) Carry out tests for humoral and cellular immunity using peripheral blood cells from protocol patients and control normal donors.

Progress: Since October 1978, 8 new batches of pooled vaccine have been prepared from human melanoma cell lines, UCLA SM-6, UCLA SM-12 and UCLA SM-14. All of these vaccine preparations met quality control tests and have been used in the melanoma immunotherapy protocol. In addition, 450 serum samples were frozen and stored in approximately 5,000 vials. During this period, data analysis by a variety of statistical procedures has solidified the following initial impressions of the results: (1) greater than 90% of melanoma patients receiving the melanoma vaccine generated cellular and humoral responses against one or more of the vaccine cell lines; (2) melanoma patients in remission, except for those receiving chemotherapy, have a normal capacity for generating cytotoxic cellular responses in vitro, and to this extent are not immunosuppressed; and (3) treatment of melanoma patients with a nonspecific immunostimulant, BCG, has not increased the average natural killer or K-cell activities of PBL from these patients. Additional findings are that both the average cellular immune activity and peripheral blood lymphocyte counts diminish prior to clinically detectable relapse in the group of patients receiving vaccine.

Significance to Cancer Research: The melanoma cell line vaccine will be used in a clinical immunotherapy trial.

Project Officer: Dr. John R. Wunderlich
Program: Tumor Immunology Program
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 79 Funds: $183,185

CONTRACT RESEARCH SUMMARY

Title: Rhesus Monkey Histocompatibility Studies

Principal Investigator: Dr. Bruce Maurer
Performing Organization: Litton Bionetics, Inc.
City and State: Kensington, MD

Contract Number: NO1-CB-64016
Starting Date: 11/1/75 Expiration Date: 6/30/78

Goal: To establish and maintain a colony of pedigreed rhesus monkey families for the study of the genetics of transplantation and as an eventual source of donor-recipient pairs for transplant experiments.

Approach: Sera raised by allo-immunization of selected monkeys are analyzed for serologically defined antigens by microcytotoxicity typing of families and large random unrelated populations. Monkeys are typed for MLC locus antigens by performing mixed lymphocyte cultures using responder lymphocytes primed in MLC to the MLC locus antigen of a sibling differing by one RhLA haplotype. The method of Mann et al. is used to identify B-lymphocyte alloantigens serologically. Breeding is designed to enlarge existing families of full siblings. The method of Gillis and Smith is used to establish lines of lymphocytes cytotoxic for RhLA alloantigen-bearing cells.

Progress: Analysis of about 300 alloantisera has resulted in improved definition or new identification of a total of 24 RhLA alloantigens. During the current contract year, analysis of several new sera by methods of population genetics and by absorption led to the identification of four of these 24. Optimization of a system for primed lymphocyte typing (PLT) has been completed. Four different primed lymphocytes have been shown to detect RhLA-linked determinants. Comparison of primed lymphocytes with homozygous typing cells indicates the two methods may not lead to identification of the identical determinant. Typing of a random population of monkeys with a panel of 57 sera detecting antigens exclusively present on B-lymphocytes has been completed. Seven antigens have been identified, and family studies indicate at least four are linked to RhLA. Specifically, cytotoxic cells have been maintained in culture for greater than 40 days. Various methods of conditioning medium for support of these cultures and screening of responder-stimulator pairs for long-term proliferative potential are being evaluated.

Significance to Cancer Research: Bone marrow transplantation may allow more intensive cancer chemo-, radio- or immunotherapy than is presently possible. Transplantation of other organs may be useful for replacement of tumor-ravaged organs.

Project Officer: Dr. J. R. Neefe, Jr.
Program: Tumor Immunology Program
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Studies of the Immune Response of Mice and Rats to Tumor Antigens

Principal Investigator: Dr. Martin Padarathsingh
Performing Organization: Litton Bionetics, Inc.
City and State: Kensington, MD

Contract Number: NO1-CB-74093
Starting Date: 2/28/77 Expiration Date: 2/27/80

Goal: Study of natural cell-mediated cytotoxicity of mice and rats and of their immune responses to tumor-associated antigens. Studies on in vivo resistance to tumors and correlation with in vitro cellular immune responses.

Approach: To study (1) the characteristics of the cellular immune responses of tumor-associated antigens to tumors induced by murine leukemia viruses and murine sarcoma virus, (2) the natural cell-mediated cytotoxicity against tumors of mice and rats, and (3) the factors influencing the levels of this reactivity and the mechanism of cytotoxicity.

Progress: The main emphasis continues to be on natural cell-mediated cytotoxicity in mice. A series of studies have been performed to study the effects of interferon on natural killer cells. The boosting by a variety of agents in vivo was shown to correlate closely with the time of appearance of circulating levels of interferon. Boosting of NK cell activity by various tumor cells could be accounted for by the ability of some tumor cells to rapidly induce circulating levels of interferon. This interferon appeared to have some characteristics of type 2 interferon, being labile at pH 2. C. parvum, another inducer of type 2 interferon, is an effective booster of NK cell activity. So it appears that both type 1 and type 2 interferon can strongly affect cytotoxic activity of NK cells. We have confirmed the reports that in vivo treatment of mice whith estradiol can cause marked reduction in NK activity and that estradiol-treated mice fail to develop augmented activity after treatment with interferon inducers. Treatment of mice with estradiol may provide a useful model for examining the in vivo role of NK cells. Studies of the possible in vivo role for NK cells were initiated. Isotopically labeled tumor cells were injected intravenously and their rate of clearance from the lungs, liver, and spleen was determined during the first 4 hours. Clearance of the labeled tumor cells has correlated fairly well with known levels of spontaneous NK activity and with the known effects of various agents in boosting or depressing NK activity.

Significance to Cancer Research: Understanding the relationship of in vitro assays to host resistance of tumors is an essential element in our ability to use these assays to monitor the clinical response to immunotherapy and to understand the role of various immune mechanisms in resistance to tumor growth. Recent findings with natural cell-mediated immunity may account for a significant portion of in vivo resistance to tumor growth, and may have important implications for immune surveillance.

Project Officer: Dr. Ronald B. Herberman
Program: Tumor Immunology Program
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 79 Funds: $208,116

CONTRACT RESEARCH SUMMARY

Title: Tumor Virus Expression in Normal and Transformed Cells

Principal Investigator: Dr. Kenneth Blackman
Performing Organization: Meloy Laboratories, Inc.
City and State: Rockville, MD

Contract Number: NO1-CB-74139
Starting Date: 6/15/77 Expiration Date: 6/14/80

Goal: To study expression of tumor viruses in tissue culture and in animals.

Approach: Viral biochemical, serologic, and biological parameters are monitored in different systems to define the regulatory mechanisms of tumor virus expression and to relate the expression to tumor production.

Progress: Harvey sarcoma virus DNA has been molecularly cloned, is biologically active, and has been used to study in detail the structure of the DNA, as a source of defined sub-genomic probes, and to define the portion of the DNA necessary for the induction of transformation.

Two integrated AKR murine leukemia virus DNA genomes have been molecularly cloned from mouse cells. Both are infectious, but their specific infectivity differs. Neither viral genome is integrated next to endogenous viral DNA.

Friend murine leukemia virus DNA has been molecularly cloned. It codes for a virus which induces leukemia in newborn mice in three weeks.

Several mouse cell lines have been found which can be morphologically transformed by the bovine papilloma virus (BPV). The transformed cells are being studied for their content of BPV genetic information.

Project Officer: Dr. Douglas R. Lowy
Program: Immunology Support
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 79 Funds: $85,350

CONTRACT RESEARCH SUMMARY

Title: National Cancer Institute Immunodiagnostic Reference Center

Principal Investigator: Dr. James Harness
Performing Organization: Meloy Laboratories, Inc.
City and State: Springfield, VA

Contract Number: NO1-CB-63976
Starting Date: 7/11/78 Expiration Date: 7/10/79

Goal: To develop new assays for tumor-associated antigens, to use immunochemical techniques for the diagnosis of cancer, to evaluate humoral immune responsiveness of patients with cancer.

Approach: To use sensitive radioimmunoassays to quantitate human, monkey, and mouse alpha-fetoprotein and human chorionic gonadotropin in serum, to evaluate these assays both as diagnostic techniques for testicular, ovarian, and hepatocellular cancer, and as a means of monitoring the clinical response to therapy. Human lung tumors are being fractionated to isolate lung tumor specific antigen reactive with cancer and to establish a radioimmunoassay for this antigen. The contractor performed tests of humoral competence of patients with cancer or immunodeficiency diseases.

Progress: In a wide-ranging study of 1,000 patients with nonseminomatous germ cell tumor of the testis or hepatocellular tumors, alpha-fetoprotein and HCG assays have been shown to be of great value as an adjunct to diagnosis, in staging, and as a tool to monitor the chemotherapy of these tumors. These assays are being performed in collaboration with eight groups in monitoring the treatment of patients with hepatocellular or testicular germ cell tumors other than seminoma. In all cases in which the marker was elevated, but where there was no clinical evidence of disease, a positive marker always reflected occult residual tumor. A human lung tumor extract has been isolated and reacts with antiserum against human lung tumors but not against normal lung tissue. A radioimmunoassay has been established and is being used to detect very small quantities of this antigen in the serum of patients with lung tumors. This assay has been utilized in efforts directed towards establishing an immunodiagnostic test for human lung cancer and for monitoring the treatment of this disease. Humoral antibody assays have been established for ten antigens and these antibody assays are of critical importance in defining immune defects in patients with high incidence of neoplasia.

Significance to Cancer Research: These studies are helping to establish the usefulness of thes multiple immunodiagnostic tests in the diagnosis of cancer, or as a means of monitoring patients during and after therapy.

Project Officers: Dr. Thomas A. Waldmann, Dr. R. Michael Blaese, Dr. Robert McIntire
Program: Tumor Immunology Program
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 79 Funds: $319,738

CONTRACT RESEARCH SUMMARY

Title: Preparation of Reagent Antisera and Antigens

Principal Investigator: Dr. Howard M. Grey
Performing Organization: National Jewish Hospital
City and State: Denver, CO

Contract Number: NO1-CB-53912
Starting Date: 5/15/75 Expiration Date: 5/15/78

Goal: Preparation of monospecific fluorescent-isomer conjugated IgG $F(ab')_2$ antibodies to all heavy and light chain classes of human and mouse immunoglobulins and human T-lymphocytes.

Approach: To purify all classes of human and mouse heavy and light chains by immunochemical techniques. To prepare solid-phase absorbed antisera to each of these antigens. The IgG fractions of such antisera will be isolated, enzyme digested to make $F(ab')_2$ fragments, and conjugated to fluorescent isomers. A variety of cell types will be used for both immunization and absorption to make human T-cell-specific antisera. The antibodies will then be further processed as for the anti-Ig reagents.

Progress: Whole rabbit antisera (approximately 100 ml) with specificity for each of the human immunoglobulins (including antisera with λ and κ specificity) have been prepared. Similarly, antisera with specificity for each of the mouse immunoglobulins, including IgG, IgG_1, IgG_2 and IgG_3 as well as mouse λ chain, and antisera with specificity for mouse IgG_2 prepared against myeloma PC5 having κ specificity have been prepared.

A variety of $F(ab')_2$ fluorescein-labeled rabbit antisera with specificity for human and mouse immunoglobulins were prepared. This completes the workscope of the contract with the following exceptions: a) additional amounts of anti-human IgM, IgA and IgD are required to bring the amount of each of these antisera supplied to approximately 75 mg; b) small amounts of each of the purified human immunoglobulins are to be supplied; c) T-cell-specific antisera are to be supplied. To date, rabbits have been immunized with monkey thymus cells. The resultant antiserum has been subjected to DEAE cellulose chromatography and the IgG obtained has been fluoresceinated. The purified, labeled antiserum thus obtained was then absorbed with RBC stroma, CLL cells and lymphoblastoid cell lines to obtain an antiserum that reacts only with a portion of human peripheral blood cells. Further work must be done to assure that the antiserum obtained is in fact T-cell specific.

Significance to Cancer Research: This contract will provide specialized antisera which are required for cell surface studies important in the Tumor Immunology Program.

Project Officers: Dr. Howard B. Dickler and Dr. Warren Strober
Program: Tumor Immunology Program
Technical Review Group: Immunology Support Contract (Ad Hoc Review)
FY 79 Funds: 0

CANCER DIAGNOSIS CONTRACT PROGRAM

CONTRACT RESEARCH SUMMARY

Title: Standard Protocol for Evaluation of Imaging Techniques in Cancer Diagnosis

Principal Investigator: Dr. John A. Swets
Performing Organization: Bolt, Beranek and Newman
City and State: Cambridge, MA

Contract Number: NO1-CB-64010
Starting Date: 6/10/76 Expiration Date: 9/9/79

Goal: To establish a new general protocol for comparison and evaluation of imaging techniques used in cancer diagnosis in order to contribute to the improved recognition of earlier cancer pathology.

Approach: The protocol will be developed to specify (1) the psychophysical procedures that provide unbiased measures of the diagnostic accuracy provided by each imaging technique, and (2) the data analyses that provide appropriate measures of the medical efficacy as well as the cost-benefit relationships associated with an imaging technique. To aid in the standard protocol development, a pilot study will be made involving an exploratory analysis of data from two collaborative studies sponsored by NCI. During the second year the protocol will be used to accomplish complete analysis of the two sets of NCI imaging data.

Progress: The standard protocol has been developed and tested in a pilot study. Because of unanticipated difficulty in obtaining necessary data required for the pilot study from the two collaborative studies, the pilot study of the standard protocol was delayed. Nearly final revisions of the standard protocol have been made. Use has been made of the standard protocol for comparison and evaluation of the imaging techniques of X-ray CT scanning and radionuclide imaging used for brain tumor detection. A similar comparison of breast cancer diagnosis using X-ray film mammography and xeromammography also has been completed. Final adjustments of the standard protocol are being made based on experience from the two studies. The standard protocol in its final form will be submitted to the Diagnosis Branch by BBN by 9 June 1979.

Significance to Cancer Research: The standard protocol will facilitate evaluation of imaging techniques used for cancer diagnosis.

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 79 Funds: $73,715

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of CTT in Brain Tumor Diagnosis

Principal Investigator: Dr. Sadek K. Hilal
Performing Organization: Columbia-Presbyterian Hospital
City and State: New York, NY

Contract Number: NO1-CB-43910
Starting Date: 6/30/74 Expiration Date: 12/29/79

Goal: To determine the value of the EMI apparatus in the diagnosis of tumors of the brain, especially as compared to nuclide scanning, angiography, and pneumoencephalography.

Approach: Five cooperating institutions will evaluate this instrument by a common protocol and forms. Patients to be studied will be divided into two groups. The first group includes those with signs and symptoms of intracranial mass lesions. This group will be further subdivided into subgroups where the probability of tumor is high, moderate, or low. The second group of patients will include those with primary malignant neoplasms of the lung or breast and those with malignant melanomas and sarcomas without neurological evidence of a brain lesion or lesions. Normal persons will also be studied by computerized tomography to give information about the X-ray absorption characteristics of the normal brain. The number of patients available in this study will provide statistically significant information.

Progress: Patient enrollment has been completed, and the only remaining work will be continuing patient follow-up until the end of 1979. Analysis and interpretation of accumulated data are being actively pursued.

Significance to Cancer Research: This collaborative study will help to establish the value of X-ray CT scanning in the detection and localization of brain tumors.

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 79 Funds: $15,954

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of CTT in Brain Tumor Diagnosis

Principal Investigator: Dr. D. Gordon Potts
Performing Organization: Cornell University Medical School
City and State: New York, NY

Contract Number: NO1-CB-43980
Starting Date: 6/30/74 Expiration Date: 12/29/79

Goal: To determine the value of the EMI apparatus in the diagnosis of tumors of the brain, especially as compared to nuclide brain scanning, angiography, and pneumoencephalography.

Approach: Five cooperating institutions will evaluate this instrument by a common protocol and forms. Patients to be studied will be divided into two groups. The first group includes those with signs and symptoms of intracranial mass lesions. This group will be further subdivided into subgroups where the probability of tumor is high, moderate, or low. The second group of patients will include those with primary malignant neoplasms of the lung or breast and those with malignant melanomas and sarcomas without neurological evidence of a brain lesion or lesions. Normal persons will also be studied by computerized tomography to give information about the X-ray absorption characteristics of the normal brain. The number of patients available in this study will provide statistically significant information.

Progress: Giant histological brain sections have been prepared on patients coming to autopsy to show the entire brain in the planes of the EMI sections; 121 cases have been studied in this manner. This will permit an accurate comparison of the X-ray CT scan views with the other studies.

Patient enrollment has been completed and the only remaining work will be continuing patient follow-up until the end of 1979. Analysis and interpretation of accumulated data are being actively pursued.

Significance to Cancer Research: This collaborative study will help to establish the value of X-ray CT scanning in the detection and localization of brain tumors.

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Algorithms for Computerized Transaxial Nuclide Reconstruction

Principal Investigator: Dr. T. F. Budinger
Performing Organization: DOE--Lawrence Berkeley Laboratory
City and State: Berkeley, CA

Contract Number: YO1-CB-50304
Starting Date: 6/30/75 Expiration Date: 6/30/79

Goal: To improve detection and diagnosis of early cancer lesions using radioactive compounds.

Approach: Hardware and software for CT nuclide reconstruction based on transverse section emission data from human subjects are in current development in numerous institutions. Such procedures appear particularly promising for use with structure-specific labeling with a wide array of radioactive compounds. Emission imaging may permit not only static reconstruction but also quantitative estimates of time-course movements of labeled compounds through structures located deep within the body. Transaxial reconstruction will permit location of radioactivity in small body volumes that would be obscured by surrounding radioactivity and internal radioabsorption in conventional viewing techniques. Algorithms will be developed for computerized reconstruction of clinical data produced by transaxial nuclide projection machines. In addition, techniques for algorithm evaluation and comparison will be developed and applied. Algorithms, written in a high level programming language such as FORTRAN, together with phantom, animal and human emission and transmission projection data, and reconstructions will be recorded on nine track industry compatible magnetic tape and delivered to the NCI. The NCI shall make independent evaluations and disseminate this information to other investigators.

Progress: The final form of the User's Manual was completed in November 1977 and made available to interested workers. It contains 285 pages of descriptive material and complete FORTRAN listings with appropriate examples of each major algorithm and modifications. Work in the final year of the contract was concentrated on continued development of (1) a method for calculating dynamic functional images by computations in projection space; (2) a new general algorithm incorporating a priori information; (3) a new approach to the attenuation problem; (4) fan beam response characterizations; (5) a hardwired FFT design for iterative reconstruction; and (6) additional development of the previously studied iterative hardwired reconstructor.

Significance to Cancer Research: This and other similar studies will improve nuclear medicine imaging capabilities for cancer detection.

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Exploration of the Use of a Proton Beam in Tissue Densitometry

Principal Investigator: Dr. Cornelius A. Tobias
Performing Organization: DOE--Lawrence Berkeley Laboratory
City and State: Berkeley, CA

Contract Number: YO1-CB-40302
Starting Date: 6/30/74 Expiration Date: 6/29/79

Goal: To evaluate the diagnostic potential of accelerated proton and various heavy ion beams in the detection of tissue abnormalities poorly or not detectable with X-rays; to develop techniques for the use of the particle beam of choice as a low dose adjunct to X-ray diagnostics.

Approach: With suitable phantoms and in vivo and in vitro biological specimens, the resolution and density detection sensitivity of various accelerated charged particle beams (H, He, C, O, Ne) will be compared using the LBL Bevatron/Bevalac for $Z \geq 2$ beams and the Harvard cyclotron for proton beams. In addition, the importance of beam energy and suitability of various detectors (such as plastic nuclear track detectors and photographic film) will be evaluated for the optimum beam particle-energy-detector system. Human diagnostic studies in cancer detection will be conducted with the system of choice. Comparisons of particle radiography with other noninvasive diagnostic techniques and pathology (when possible) will be made.

Progress: Studies to date, with phantoms and biological targets (including freshly excised mammary specimens), indicate that radiography with heavy ion beams (C, O, Ne) using a multi-layered stack of plastic foils for image registration affords substantially better lateral and depth resolution than light ion beams (proton, alpha) using a stack of photographic film. The heavy ion/plastics system produces superior depth resolution but inferior lateral resolution when compared with a conventional X-ray/film system. Techniques for low dose (0.1 rad) patient carbon mammography and extremity radiography have been developed and patients radiographed with promising results. Tumor structures in the human breast invisible to X-rays have now been seen with heavy ions. Imaging of the head and abdomen has also been studied in a few cases. Initial work in optical and computer synthesis of the images from a single multi-layered plastic stack into a single composite image has been done with encouraging results. During the final year of the contract work was concentrated on heavy ion imaging studies of (1) cancer patients with emphasis on soft tissue; (2) isolated tissues and organs; and (3) micro-beam histological studies.

Significance to Cancer Research: Results from this study will help define the possible diagnostic usefulness of heavy ion beams in cancer.

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Screening for Medullary Carcinoma of the Thyroid Using Calcitonin Radioimmunoassay

Principal Investigator: Dr. Samuel A. Wells
Performing Organization: Duke University
City and State: Durham, NC

Contract Number: NO1-CB-63994
Starting Date: 6/15/76 Expiration Date: 6/14/81

Goal: The goal of this project is to establish a periodic screening program of relatives of patients with medullary carcinoma of the thyroid gland (MCT).

Approach: A cohort of patients in which MCT is proven histologically will be established. The cohort will be sufficiently large to offer a statistically significant number of family members. Those family members will be screened for serum calcitonin levels by radioimmunoassay methods in order to find MCT during its early stage of development. A long-term follow-up program will be conducted on these families to determine the natural history of the disease and effectiveness of therapy.

Progress: Our studies of approximately 700 subjects from seven kindreds with multiple endocrine neoplasia, type II, (medullary thyroid carcinoma (MTC), pheochromocytoma and parathyroid hyperplasia) has completed its third year. We have demonstrated that the combined infusion of calcium gluconate (2 mg/kg/1 minute) followed by pentagastrin (0.5 µg/kg/5 seconds) is the preferred method of stimulating calcitonin (CT) release from MTC cells. Patients with occult MTC who are diagnosed biochemically following provocative testing are younger, have a lower incidence of regional node metastases, and have a lower incidence of elevated plasma CT levels following provocative testing post-operatively. Plasma samples from selectively catheterized inferior thyroid veins following provocative testing have yielded strikingly higher CT values than in concomitantly-sampled peripheral blood. This technique of selective catheterization has been especially useful in diagnosing MEN-II kindred members at risk whose peripheral plasma CT levels are undetectable basally, but are moderately increased (250-1000 picograms/ml) following provocative testing.

Project Officer: Louis P. Greenberg
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 79 Funds: $95,000

CONTRACT RESEARCH SUMMARY

Title: Development of Large Area Solid State Image Receptors for X-ray Imaging

Principal Investigator: Dr. Alan R. Sears
Performing Organization: General Electric Co.
City and State: Schenectady, NY

Contract Number: NO1-CB-74112
Starting Date: 9/30/77 Expiration Date: 9/29/79

Goal: To improve clinical diagnostic radiology, for use in diagnosis of early cancer, by developing an X-ray sensor superior in clinical usefulness to those currently available.

Approach: A large area solid state X-ray detector will be produced which will transform the X-ray flux into a digitally stored high quality image. The system to be developed consists of two critical components, an X-ray absorbing electrophoretic image display cell and a scanning system, which are coupled with the necessary control, display, and image storage memory components. The critical components will be developed during the first year and their feasibility demonstrated. Further development and optimization of the critical components and the assembly of the remaining components into the finished system will be completed during the second year. Clinical evaluation of the system will be carried out during the third year.

Progress: The key component requiring the most basic research and development effort in this innovative system was the X-ray absorbing electrophoretic image display cell. During the initial year of the work the problems involved in this cell were well defined and work during the second year has been concentrated on solving those problems. Good progress was made in the second year, but an imaging cell with the superior imaging quality required for use with the rest of the system was not yet produced. This problem has caused essentially two years delay in the original estimate of the total time required to produce a prototype system and to test its clinical feasibility. Accordingly, the contract will not be continued beyond 29 September 1979.

Significance to Cancer Research: This system will allow digital storage, transmission, retrieval, and selection of imaging information.

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of CTT in Brain Tumor Diagnosis

Principal Investigator: Dr. David O. Davis
Performing Organization: George Washington University
City and State: Washington, DC

Contract Number: NO1-CB-43981
Starting Date: 6/30/74 Expiration Date: 12/29/79

Goal: To determine the value of the EMI apparatus in the diagnosis of tumors of the brain, especially as compared to nuclide brain scanning, angiography, and pneumoencephalography.

Approach: Five cooperating institutions will evaluate this instrument by a common protocol and forms. Patients to be studied will be divided into two groups. The first group includes those with signs and symptoms of intracranial mass lesions. This group will be further subdivided into subgroups where the probability of tumor is high, moderate, or low. The second group of patients will include those with primary malignant neoplasms of the lung or breast and those with malignant melanomas and sarcomas without neurological evidence of a brain lesion or lesions. Normal persons will also be studied by computerized tomography to give information about the X-ray absorption characteristics of the normal brain. The number of patients available in this study will provide statistically significant information.

Progress: Patient enrollment has been completed, and the only remaining work will be continuing patient follow-up until the end of 1979. Analysis and interpretation of accumulated data are being actively pursued.

Significance to Cancer Research: This collaborative study will help to establish the value of X-ray CT scanning in the detection and localization of brain tumors.

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 79 Funds: $14,279

CONTRACT RESEARCH SUMMARY

Title: Hormone Markers for the Detection and Diagnosis of Cancer

Principal Investigator: Dr. William D. Odell
Performing Organization: Harbor General Hospital
City and State: Torrance, CA

Contract Number: NO1-CB-43903
Starting Date: 9/1/78 Expiration Date: 8/31/79

Goal: To develop hormone markers that might indicate the presence of small (early), treatable cancer.

Approach: (1) To measure ADH, vasotocin, oxytocin, α and β MSH, ACTH, α and β TSH, and α and β hCG to search for tumor elaboration of these peptides among cancer patients; (2) to develop new techniques for separating and identifying "prohormone" forms of ACTH, gastrin, and pancreozymin-cholecystokinin (PZ-CCK); (3) to search for the existence of such big forms of ADH, oxytocin, α and β MSH, luteinizing hormone, and α and β glycopeptides; (4) to develop peptide assays for fetal hormones related to vasopressin; and (5) to identify other nonhormonal peptides which might be used as cancer markers. The contractor hypothesized that "ectopic peptide elaboration is a universal concomitant of neoplasia." This hypothesis is based on a review of several hormonal syndromes produced by nonendocrine cancers and the fact that, where known, they are produced by peptides. Production of nonbiologically active peptides is even more common than production of active peptides.

Progress: This contract supported a series of fundamental and clinical studies related to peptides or protein elaboration by cancers. Based upon (1) the breadth of expression the humoral syndromes that were known to be associated with cancers and (2) the fact that, where understood, with a single exception, these syndromes were caused by protein elaboration by cancer, we postulated in our proposal for this contract that "Ectopic protein elaboration is a universal concomitant of neoplasia." Using either existing assays previously developed in our laboratories (vasopressin radioimmunoassays, radioreceptor assay for ACTH, radioimmunoassay for free alpha chain of hCG) or assays developed as part of this contract (radioimmunoassays for gonadotropin-releasing hormones, for calcitonin, for ACTH, for melanocyte-stimulating hormone, endorphins), we performed both retrospective and blind prospective clinical studies. Patients with known cancers and selected diseases that might mimic cancers, and healthy populations were studied in retrospective studies. Patients admitted to the hospital with abnormal chest X-rays or gastrointestinal bleeding were studied in blind prospective studies. Cancer tissues and normal tissues were obtained at postmortem or surgery and were extracted. Extracts were analyzed for hormonal peptides, and the biochemical characteristics of these peptides were determined. Approximately 4,000 patients were studied, and data were organized using computerized analysis with individual patient chart search.

Project Officer: Louis P. Greenberg
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Early Detection of Ovarian Carcinoma in Asymptomatic Women

Principal Investigator: Dr. Malaya Bhattacharya
Performing Organization: Health Research, Inc.
City and State: Buffalo, NY

Contract Number: NO1-CB-64013
Starting Date: 6/30/76 Expiration Date: 6/29/79

Goal: The goal of this project is the development of sensitive immunoassay procedures for early diagnosis of ovarian cancers and serial monitoring of patients undergoing therapy for ovarian cancers.

Approach: The investigation will be conducted on the following topics: (1) preparation of antisera (anticystadenocarcinoma sera); (2) sources of ovarian cystadenocarcinoma-associated antigens (OCAA); (3) purification of OCAA by conventional methods, immunoadsorbent columns, affinity columns of plant lectins; (4) determination of purity of OCAA; (5) characterization of OCAA; (6) determination of the frequency of occurrence of OCAA in other cancer and non-cancer tissues; (7) development of a radioimmunoassay for detection of OCAA; (8) patient selection, availability, and follow-up; (9) characterization of immune response to ovarian cancers with emphasis on cystadenocarcinoma; and (10) problems and limitations of the proposed projects.

Progress: Ovarian cystadenocarcinoma-associated carcinoma, OCAA, is comprised of several antigenic variants. We have isolated a different molecular species and subspecies of OCAA from two large specimens of ovarian tumors which were potentially rich in OCAA. Antisera have been raised in rabbits against each of them. We will develop radioimmunoassays for their detection in patient sera. A double antibody radioimmunoassay has already been developed for one of the variants, namely OCAA-QAE-sephadex-peak-1. The cut-off point used is 5 nanograms per ml of serum. This assay seems to be useful for differential diagnosis of ovarian cancer, since elevated levels are not seen with benign ovarian cancer patients or normal age-matched healthy women. Preliminary results on serial determination on ovarian cancer patients' sera appear promising; however, a detailed clinical trial including a large group of patients is now under way to evaluate the assay.

The second ovarian cancer antigen, OCAA-1, has been purified considerably using antibody agarose and lectin agarose columns.

Project Officer: Louis P. Greenberg
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Lung Cancer Control: Detection and Treatment

Principal Investigator: Dr. John K. Frost
Performing Organization: Johns Hopkins University
City and State: Baltimore, MD

Contract Number: NO1-CB-45037
Starting Date: 9/1/78 Expiration Date: 7/15/80

Goal: To determine the effect of the early detection of lung cancer by sputum cytology, followed by surgical removal of the tumor on long-term survival in the disease (Phase II of Hopkins Study).

Approach: The study population consists of males, 45 years or older, who smoke a pack or more of cigarettes a day. By random assignment of individuals responding to advertisement, it was planned to accumulate over several years two groups of over 5,000 each. The test group would receive sputum cytologic examination and chest X-ray every four months; the control group will have an annual chest X-ray. Subjects suspicious or positive for cancer would be carefully worked up, to include fiberoptic bronchoscopy; cancers would be removed surgically. All subjects will be followed for at least 5 years to determine actuarial survival.

Progress: A total of 10,390 men have been enrolled in this project, of which 5,163 were randomized into an "X" Control Group and 5,227 into a "CX" Study Group. To date, 344 have died, while 582 have withdrawn from the study, and 89 have moved from the area. Currently, 9,144 remain as active participants; 4,578 in the "X" Group and 4,566 in the "CX" Group. At the initial screening 73 cancers were detected (38 in the X group and 35 in the CX group) for a detected prevalence of 7.0 per 1,000. Between the initial negative screening and the first annual examination a total of 87 cancers appeared (45 in the X group and 42 in the CX group) for an incidence of 8.4 per 1,000. In the CX group there were 27 cases detected with 7 (26%) Stage 0 and 10 (37%) Stage I. Fifteen additional cases occurred in the CX group between screening procedures. In the X group 33 cases were detected with none in Stage 0 and 15 (46%) Stage I. Twelve additional cases occurred in the X group between screenings. Among the 27 cases detected in the CX group 18 were resected; 20 of the 33 detected in the X group were resected. Total cancers appearing through the first annual screening were 160 with 83 in the X group and 77 in the CX group. Among the 83 found in the X group 52 (63%) have died; 37 (48%) of the 77 in the CX group have died. In terms of lung cancer deaths per 10,000 person-years of observation the rates are 32 in the X group and 22 in the CX group.

Significance to Cancer Research: Early cytologic detection, the best present Hope for improving lung cancer mortality, requires rigid proof of its effectiveness.

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 79 Funds: $930,586

CONTRACT RESEARCH SUMMARY

Title: Study of Innovative Techniques To Facilitate Passage of Colonoscope to the Cecum

Principal Investigator: Dr. John A. Coller
Performing Organization: Lahey Clinic Foundation
City and State: Boston, MA

Contract Number: NO1-CB-74212
Starting Date: 9/26/77 Expiration Date: 9/25/80

Goal: To develop a means for improving early detection of small cancer lesions on endoscopically approachable surfaces of the colon in order to improve life expectancy of the postoperative patient.

Approach: A colonoscope advancing device will be developed to enhance the ease and speed of passage of currently available colonoscopes. Modeling studies and bench testing will be conducted during the initial year to determine which of several possible designs may be feasible. Further development of the colonoscope advancing device design will be carried out during the second year aided by physiological studies made to characterize as many physical parameters of the human colon as possible. Promising devices will be given preliminary clinical tests during the latter part of the second year. After further optimization the clinical prototype propulsive devices will be tested by clinical trials in the third year.

Progress: Model development, mathematical modeling, prototype development, and bench testing performed in the initial year have demonstrated feasibility of some of the proposed devices and provided part of the background information necessary for the development of a workable prototype propulsive device during the second year. Both active and passive inflatable devices, to be attached to colonoscopes to facilitate passage, are being studied. During the second year development of fabrication techniques and investigation of various materials for producing the prototype devices are progressing. Animal studies have been added to the original plan of attack to aid both in design and preliminary testing of new models.

Significance to Cancer Research: This work is expected to provide improved transit of the entire colon by the colonoscope and, therefore, improve viewing of the colon surface for early cancer lesions.

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 79 Funds: $143,480

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of CTT in Brain Tumor Diagnosis

Principal Investigator: Dr. Paul F. J. New
Performing Organization: Massachusetts General Hospital
City and State: Boston, MA

Contract Number: NO1-CB-43983
Starting Date: 6/30/74 Expiration Date: 12/29/79

Goal: To determine the value of the EMI apparatus in the diagnosis of tumors of the brain, especially as compared to nuclide brain scanning, angiography, and pneumoencephalography.

Approach: Five cooperating institutions will evaluate this instrument by a common protocol and forms. Patients to be studied will be divided into two groups. The first group includes those with signs and symptoms of intracranial mass lesions. This group will be further subdivided into subgroups where the probability of tumor is high, moderate, or low. The second group of patients will include those with primary malignant neoplasms of the lung or breast and those with malignant melanomas and sarcomas without neurological evidence of a brain lesion or lesions. Normal persons will also be studied by computerized tomography to give information about the X-ray absorption characteristics of normal brain. The number of patients available in this study will provide statistically significant information.

Progress: Patient enrollment has been completed, and the only remaining work will be continuing patient follow-up until the end of 1979. Analysis and interpretation of accumulated data are being actively pursued.

Significance to Cancer Research: This collaborative study will help to establish the value of X-ray CT scanning in the detection and localization of brain tumors.

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Clinical Evaluation of CTT in Brain Tumor Diagnosis

Principal Investigator: Dr. Hillier L. Baker, Jr.
Performing Organization: Mayo Foundation
City and State: Rochester, MN

Contract Number: NO1-CB-43982
Starting Date: 6/15/74 Expiration Date: 12/29/79

Goal: To determine the value of the EMI apparatus in the diagnosis of tumors of the brain, especially as compared to nuclide brain scanning, angiography, and pneumoencephalography.

Approach: Five cooperating institutions will evaluate this instrument by a common protocol and forms. Patients to be studied will be divided into two groups. The first group includes those with signs and symptoms of intracranial mass lesions. This group will be further subdivided into subgroups where the probability of tumor is high, moderate, or low. The second group of patients will include those with primary malignant neoplasms of the lung or breast and those with malignant melanomas and sarcomas without neurological evidence of a brain lesion or lesions. Normal persons will also be studied by computerized tomography to give information about the X-ray absorption characteristics of the normal brain. The number of patients available in this study will provide statistically significant information.

Progress: Patient enrollment has been completed, and the only remaining work will be continuing patient follow-up until the end of 1979. Analysis and interpretation of accumulated data are being actively pursued.

Significance to Cancer Research: This collaborative study will help to establish the value of X-ray CT scanning in the detection and localization of brain tumors.

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 79 Funds: $12,400

CONTRACT RESEARCH SUMMARY

Title: Mayo Lung Project for Detection and Localization of Early Lung Cancer

Principal Investigator: Dr. Robert S. Fontana
Performing Organization: Mayo Foundation
City and State: Rochester, MN

Contract Number: NO1-CB-53886
Starting Date: 10/1/74 Expiration Date: 10/1/80

Goal: To test new methods of diagnosis of early lung cancer and to assess survival of patients with lung cancer detected by these methods.

Approach: A study population of 9,223 subjects was obtained following initial screening of 11,001 non-volunteers. Candidates for the study were Mayo Clinic outpatients who were men, 45 years old or more, smoking one pack of cigarettes or more daily. None of those accepted into the study had a history or suspicion of respiratory cancer on entry into the Clinic; all had a life expectancy of at least 5 years, and all were judged capable of tolerating pulmonary resection (at least lobectomy). The initial screening consisted of chest X-rays, 3-day sputum cytology tests and lung health questionnaires. This screening yielded 87 unsuspected ("prevalence") lung cancers, 16 detected by cytology alone, 54 by X-ray alone, and 17 by both tests. Radiographically "occult" cancers were localized fiberbronchoscopically. The 9,223 study subjects who had "negative" initial screens were randomized into a close surveillance group, rescreened every 4 months, and a standard surveillance ("control") group advised to undergo rescreening once a year. A separate population of post-surgical AJC stage I lung cancer patients is also being evaluated, as are hematoporphyrin derivative (HpD) and cryotherapy as techniques for "mapping" and treating carcinoma-in-situ.

Progress: As of April 1, 1979, both the 4-monthly surveillance group and the "control" group had been observed nearly 20,000 man-years, and 183 new ("incidence") cases of cancer of the respiratory tract had been detected, 40 involving the upper airway and 143 the lungs. In the 4-monthly surveillance group there were 86 new lung cancers (12 of which were detected by cytology only) and 36 lung cancer deaths. Early survival data in this group are encouraging. In the control group there were 57 new lung cancers and 39 lung cancer deaths, with a tendency toward more traditional lung cancer survivorship. Thus, once lung cancer has been detected, survival in the 4-monthly surveillance group is considerably better than among the controls. Yet so far no significant decrease in total death rate from lung cancer has been observed in the close-surveillance group. However, as duration of observation has increased, the yearly lung cancer death rates in the control group have first equalled and then exceeded corresponding levels for the close surveillance group. Longer observation and prolonged follow-up are essential before final conclusions and specific recommendations will be possible.

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
Relevance Review Group: Institute Director and Executive Committee
FY 79 Funds: $768,090

CONTRACT RESEARCH SUMMARY

Title: Usefulness of Carcinoembryonic Antigen in the Diagnosis of Bowel Cancer

Principal Investigator: Dr. Vay Liang W. Go
Performing Organization: Mayo Foundation
City and State: Rochester, MN

Contract Number: NO1-CB-23854
Starting Date: 6/15/72 Expiration Date: 6/14/79

Goal: (a) Measure CEA levels in blood and other specimens stored at NCI-Mayo Clinic Tumor Plasma Bank (CB-23879), Mayo Lung Cancer Project (NCI-CB-22000) and other contracts with the Immunodiagnosis Program of the Division of Cancer Biology and Diagnosis; (b) continue to collect and provide colonic malignant tissue for different investigators in projects dealing with carcinoembryonic antigens; (c) to complete the work done over the last 4 years: (1) evaluate the CEA test in diagnosis of large bowel carcinoma; (2) continue evaluation of CEA as a biologic marker in patients undergoing chemotherapy, radiotherapy, and immunotherapy of advanced gastrointestinal cancer; and (3) immunological quantification and chemical characterization of CEA-like material in secretions of the gastrointestinal tract.

Progress: (a) Progress related to the contract's service function activity. Serum or plasma levels have been determined in 8,211 specimens from NCI Serum Plasma Bank including those sent in from other investigators. These samples were run at 20% of the commercial cost. The comparative value of CEA to other tumor-associated antigens was done with other investigators from other institutions. Statistical analyses of the data over the last three years were included in the Workshop on Immunodiagnosis of Human Cancer Statistical Analyses, Part II. Results and statistical analyses are being performed on coded serum panels sent to many investigators with potentially useful diagnostic tests for cancer.

(b) Progress report on the evaluation of CEA as a biological marker. The collection of data has now been completed for the project in the evaluation of CEA as a biological marker in patients undergoing chemotherapy, radiotherapy, and immunotherapy for advanced gastrointestinal cancer and also in patients following surgical resection of gastrointestinal cancer with curative intent and lung cancer. These data are currently being analyzed prior to publication.

Project Officer: Louis P. Greenberg
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 79 Funds: $54,000

CONTRACT RESEARCH SUMMARY

Title: Reconstruction Algorithms for Dose Reduction in X-Ray Computed Tomography

Principal Investigator: Dr. Richard A. Robb
Performing Organization: Mayo Foundation
City and State: Rochester, MN

Contract Number: NO1-CB-84235
Starting Date: 9/29/78 Expiration Date: 9/28/80

Goal: To reduce, by means of improved reconstruction methods, X-ray dosage required for use in the CT scanning procedures used in cancer diagnosis.

Approach: A variety of approaches will be investigated for their possible contributions to improved image reconstruction with reduction in required X-ray dosage. These will include studies for optimizing convolution, least square, and iterative algorithms for X-ray dose reduction; use of a priori information; noise reduction algorithms; variance maps for regional reconstruction; limitation of ranges, numbers of views, and field of view; nonlinear spatial smoothing; and optimizing algorithms based on multiplier methods, penalty functions, and multiple criteria optimization.

Progress: Preliminary studies are being carried out during this initial period of the contract.

Significance to Cancer Research: This work will provide means to achieve cancer diagnosis at lower radiation risk.

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnosis Special Review Group
FY 79 Funds: $113,577

CONTRACT RESEARCH SUMMARY

Title: Lung Cancer--Early Detection, Localization, and Therapy

Principal Investigator: Dr. Myron R. Melamed
Performing Organization: Memorial Hospital
City and State: New York, NY

Contract Number: N01-CB-45007
Starting Date: 9/1/73 Expiration Date: 8/31/80

Goal: To evaluate sputum cytopathology as a supplement to annual chest X-rays in detecting pulmonary neoplasms at an "early stage" and to evaluate the efficacy of techniques for prompt localization of radiologically occult lung cancer (e.g., before progression to X-ray positive); in general, to evaluate the efficacy of such screening to reduce lung cancer mortality.

Approach: Over a 3-year period, 5,000 test subjects and 5,000 control subjects will be entered into this study with active screening for 5 years, all to be followed for at least an additional 5-year period, for sufficient years to be statistically significant to answer pertinent questions. This will be conducted according to a protocol developed in conjunction with the Johns Hopkins University and the Mayo Foundation.

Progress: Recruitment for the Memorial Sloan-Kettering Cancer Center National Lung Program was completed in January 1978. Total enrollment is 10,040. Participants were randomly assigned to study group A (4,969), receiving annual chest X-rays and 4-monthly sputum; and to control group B (5,071), receiving annual X-rays only. There have been a total of 132 confirmed lung cancers identified so far, 74 in group A and 58 in group B. The principal mode of detection in the A group was by cytology in 16 cases, X-ray in 39 cases and both techniques in 7 cases. Of the 16 cases detected by cytology, 10 were Stage 0 or I; of the 46 cases detected by radiology (or radiology and cytology) in group A and the 42 cases detected by radiology in group B, a total of 88 cases, there were 39 in Stage I. There were 28 interval cancers diagnosed following symptoms or signs, and 15 were oat cell cancers.

Among the 74 lung cancers appearing in group A, 32 were prevalence cancers and 42 were incidence cancers; 17 of the 32 and 19 of the 42 have died. In group B the 58 lung cancers included 21 prevalence and 37 incidence cases; 13 of the 21 and 15 of the 37 have died. In terms of total deaths from lung cancer per 10,000 person-years of observation the rates are 22 in study group A and 19 in control group B. For incidence lung cancer deaths the respective rates are 13 and 11.

Significance to Cancer Research: Early cytologic detection, the best present hope for improving lung cancer mortality, requires rigid proof of its effectiveness.

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 79 Funds: $850,000

CONTRACT RESEARCH SUMMARY

Title: Methods of Testing Effect of Screening for Endometrial Cancers by Uterine Samples

Principal Investigator: Dr. Leopold Koss
Performing Organization: Montefiore Hospital
City and State: New York, NY

Contract Number: NO1-CB-84233
Starting Date: 9/30/78 Expiration Date: 9/29/80

Goal: To carry out an extensive study to test the feasibility of screening for endometrial cancers in asymptomatic women by means of uterine sampling.

Approach: Subjects for the study will be selected from a group of women over age 45 and up to age 70 years that regularly attend the clinic of the contractor's institution. From the group, by appropriate means outlined below, 3,200 women will be enrolled into the study. Sixteen hundred of the women will be in the age range 46 to 59 years and 1,600 will be in the age range of 60 to 70 years. After history has been obtained and the examining procedure explained, each consenting examinee will receive a complete gynecologic examination, a vaginal pool smear, a cervical scrape smear, and an endocervical aspiration smear. Subsequently the examinees whose clinic numbers are odd will receive a Mimark endometrial sampling and the examinees with even clinic numbers will receive the Isaacs (Curity) endometrial sampling. The patient's acceptance of the procedure will be evaluated and recorded by the gynecologist upon completion of the examination. Results of the laboratory examination will be communicated by the contractor to the patient's primary physician and to the patient.

Progress: After a three-month period to assemble the personnel and organize the work, enrollment of subjects began in January 1979. The physical facilities were completed in early February 1979. Patient enrollment by spring 1979 was accelerating. Preliminary results on the first 150 women examined indicate successful endometrial sampling in more than 80 percent of the cases. No conclusions are available concerning relative effectiveness of the two endometrial sampling procedures.

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 79 Funds: $260,407

CONTRACT RESEARCH SUMMARY

Title: Study of Innovative Techniques To Facilitate Passage of Colonoscope to the Cecum

Principal Investigator: Dr. Max Epstein
Performing Organization: Northwestern University
City and State: Evanston, IL

Contract Number: NO1-CB-74114
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: To develop a means for improving early detection of small cancer lesions on endoscopically approachable surfaces of the colon in order to improve the life expectancy of the postoperative patient.

Approach: A colonoscope advancing device will be developed to enhance the ease and speed of passage of currently available colonoscopes. Prototype design, fabrication, and preliminary testing with simulated environment models during the initial year will be followed by animal tests of promising designs during the second year. Further development and preliminary clinical testing during the second year will lead to the clinical prototype colonoscope advancing device which will be tested by clinical trials in the third year.

Progress: Prototypes of three different devices have been produced: an endoscopic extender ("baby scope"); an inflatable liner; and a crawling sleeve. The present endoscopic extender model consists of an aligned fiber-optic bundle, 1.4 mm diameter, attached to a 1.45 mm diameter distal microlens system which provides a 63° field of view. This device has been tested successfully in dogs and will be tested soon in humans. The inflatable liner system is undergoing rapid development after successful tests of earlier models in dogs. Currently a liner of slightly smaller diameter is being constructed. A final design of the crawling sleeve has been selected and a supplier has been found to construct the mold which will be used to fabricate the sleeve. During the remainder of the second year of the contract the endoscopic extender will be tested in human subjects, the inflatable liner development will continue and final development of the crawling sleeve system will continue. Testing of the crawling sleeve system in dogs will be carried out in the summer of 1979.

Significance to Cancer Research: This work is expected to provide improved transit of the entire colon and thereby make easier the endoscopic viewing of the colon surface for early cancer lesions.

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 79 Funds: $51,173

CONTRACT RESEARCH SUMMARY

Title: Contrast Agents To Improve Detection of Small Liver Metastases with Computerized X-Ray Tomography

Principal Investigator: Dr. Douglas F. Adams
Performing Organization: Peter Bent Brigham Hospital
City and State: Boston, MA

Contract Number: NO1-CB-84234
Starting Date: 7/1/78 Expiration Date: 6/30/80

Goal: To improve detection of small metastatic lesions in the liver by improved use of contrast agents to provide appropriate image enhancement in X-ray computerized tomographic studies.

Approach: The work, which will be carried out over a three-year period, will consist of six tasks: synthesis of specific new contrast agents; preliminary evaluation in animal models; toxicity evaluation in animals; pilot clinical studies with currently available contrast agents; pilot clinical studies of promising new agents with acceptable toxicity characteristics; and clinical trials on the most promising contrast agent.

Progress: A computer model, confirmed by phantom studies, revealed that increasing background radiopacity enhances detectability of a target lesion in CT. This model is analogous to small metastatic nodules in normal hepatic parenchyma. Further computer analysis indicated that elements close to atomic number 60 displayed greatest absorption at kilovoltages commonly used in CT. Therefore suspensions of cerium, dysprosium, gadolinium, and silver iodide were prepared and evaluated in a rabbit liver V2 carcinoma model. Preliminary toxicity studies were also performed. Density of hepatic parenchyma was increased appreciably with only minimal elevation of tumor density, thus enhancing considerably detectability of V2 carcinoma metastases when compared with studies using standard intravenous iodinated contrast agents in similar doses. Lesions 4 mm or larger were easily detected.

A second line of investigation has focused on iodinated analogs of the new hepatobiliary agent HIDA. The fact that changes in the water-lipid partition coefficient dramatically affect hepatic extraction of these agents is being utilized in the design of additional derivatives. Human studies with standard i.v. contrast agents indicate that all primary tumors and metastases thus far encountered display contrast transit characteristics which differ from normal hepatic parenchyma. Thus, rapid scanning reveals data useful in differentiating tumors from normal liver, cysts, or abscesses.

Significance to Cancer Research: This work will provide means for improved diagnosis of liver cancer as well as establish usefulness of contrast agents in CT scanning of various tissues and organs.

Project Officer: R. Q. Blackwell, Ph.D.
Program: Cancer Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 79 Funds: $182,558

CONTRACT RESEARCH SUMMARY

Title: Development of Ultrasonic Probes To Be Inserted Through Endoscopes for Use in Cancer Diagnosis

Principal Investigator: Mr. Philip S. Green
Performing Organization: SRI
City and State: Menlo Park, CA

Contract Number: NO1-CB-74136
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: To establish an ultrasonic system for improving early detection of small cancer lesions located in body regions approachable by endoscopes in order to improve life expectancy of the patient after appropriate treatment of the lesion.

Approach: The endoscopic ultrasonic detector system will be developed and fabricated during the first 21 months of the study by SRI personnel with clinical consultation by co-investigators from the School of Medicine of the University of California at San Francisco. Optimization of the system will be accomplished by the personnel from both organizations during the second year. Preliminary clinical evaluation and optimization of the system at UCSF will be done in the last quarter of the second year, and full clinical trials at UCSF will be conducted during the third year.

Progress: Preliminary work was done on components of the detector system during the first year of the contract. Principal effort was directed toward development and evaluation of transducer arrays and of electronics for array control and signal processing. Mechanical design of the system was begun with particular emphasis on the bundle of coaxial cables which must be integrated with the endoscope. All components were assembled in a test-bed system. A transducer modeling program was written and used for design studies to improve transducer performance. In April 1979, the test-bed electronic system and a nonoptical ultrasonic endoscope were installed at Mayo Clinic for preliminary animal studies of pancreatic imaging. In the second half of the second year the complete endoscopic system for humans will be completed and installed at Mayo Clinic for preliminary clinical testing to prepare for the clinical testing to be done in the third, and final, year of the study.

Significance to Cancer Research: The ultrasonic probe will provide improved diagnostic capability for cancer lesions situated in body regions which can be approached by endoscopes.

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 79 Funds: $192,058

CONTRACT RESEARCH SUMMARY

Title: Early Detection of Ovarian Carcinoma in Asymptomatic Women

Principal Investigator: Dr. Leo Stolbach
Performing Organization: Tufts University
City and State: Boston, MA

Contract Number: NO1-CB-64028
Starting Date: 6/30/76 Expiration Date: 12/31/79

Goal: The goal of this project is to develop a screening test for the early diagnosis of ovarian carcinoma in asymptomatic women.

Approach: Malignant effusion of patients with ovarian carcinoma will be processed in order to (1) isolate and purify tumor-specific antibodies and/or antibody-antigen complexes from effusion fluids; (2) dissociate the antibody-antigen complexes and separate the tumor-associated antigens and antibodies making up these complexes; (3) determine the immune specificity of antibodies isolated from the effusion fluids; (4) detect tumor-associated antigen in the effusion fluid and tumor extracts; (5) utilize the tumor-specific antibodies to prepare an immunoadsorbent for the purification of the antigen; and antibodies to prepare an immunoadsorbent for the purification of the antigen; and (6) develop a diagnostic assay for detecting circulating antibody, antigen, and antigen-antibody complexes in patients with various stages of ovarian malignancy.

Progress: In the past year we have developed a microcomplement fixation assay to measure the relative amounts of ovarian cancer-associated antigen in human effusion fluids and in tumor tissue extract. Immune complexes have been isolated from these fluids, and the IGG from these complexes has been demonstrated to bind preferentially to ovarian cancer cells. This interaction can be inhibited by pre-incubation with a dissociated antigen fraction. In order to more conveniently measure the level of this protein in large numbers of samples we are currently producing antibodies to this purified antigen fraction for use in an enzyme-linked immunosorbent assay (ELISA). This sensitive assay will be used to measure the level of ovarian cancer-associated protein in serum, fluid, and tissue samples.

Project Officer: Louis P. Greenberg
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 79 Funds: $49,500

CONTRACT RESEARCH SUMMARY

Title: Radioisotope Surface Markers and Detectors for Endoscopic Techniques

Principal Investigator: Dr. James M. Woolfenden
Performing Organization: University of Arizona
City and State: Tucson, AZ

Contract Number: NO1-CB-64012
Starting Date: 6/30/76 Expiration Date: 12/29/80

Goal: To establish a means for improving early detection of small cancer lesions on endoscopically approachable surfaces in order to improve the life expectancy of the patient.

Approach: A system will be developed for endoscopic detection of small cancer lesions in mucosal surfaces by means of tumor-seeking radioisotopic markers. The system will include one or more appropriate tumor-seeking labeled markers and a detector system suitably matched to emission characteristics of the markers to provide optimum localization of lesions. During the first year work will concentrate on development and testing of tumor-seeking markers. During the second year development work on markers will continue, and testing of markers in animal models will be expanded. In addition, work will be started to develop suitable detectors. Finally any necessary additional development or modification of tumor-seeking agents will be made; development and optimization of the detector system and its incorporation into endoscopic equipment will continue; and clinical evaluation of the system using a previously agreed upon protocol will be conducted.

Progress: Radiolabeled bleomycin is being used for the tumor-seeking marker. Separation of individual cobalt-labeled bleomycin fractions by high pressure liquid chromatography is being studied. Separate fractions are being studied for relative distribution in animal tumor models and characterized by thin layer chromatography and high voltage electrophoresis. Sodium iodide and cesium iodide detectors are being characterized. A tumor phantom apparatus is being used to evaluate detector performance. Preliminary endoscopic tumor localization studies have been performed in several patients with relatively large lung tumors. The system used included a sodium iodide scintillator coupled by a flexible fiberoptic light guide to a photo-multiplier tube with its output interfaced to a multichannel analyzer. In each test the detector system performed satisfactorily with appropriate increase in count rate as the detector approached the tumor. Tumor localization was not impeded by background activity. Current work includes studies of detector response to varying tumor size, minimum tumor size for detection, and comparative performance of different detectors.

Significance to Cancer Research: This system, if successful, will allow endoscopic localization of small cancer lesions.

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Imaging Capability Improvement and Evaluation in Detecting Early Pancreatic Cancer

Principal Investigator: Dr. A. B. Moossa
Performing Organization: University of Chicago
City and State: Chicago, IL

Contract Number: NO1-CB-84232
Starting Date: 8/1/78 Expiration Date: 7/31/80

Goal: To compare, improve, and evaluate imaging methods and strategies applied to the early detection and diagnosis of pancreatic cancer.

Approach: The imaging methods and strategies will be applied to the study of a selected population of patients in whom there is a high clinical probability of pancreatic cancer. The group will include patients with pathological conditions not related to the pancreas, patients with benign pancreatic disease, and patients with pancreatic cancer. The purpose of the study is to improve the ability of the diagnostic team to achieve rapid and certain identification of the patients with early pancreatic cancer within the high probability group of patients. Patients must be over 35 years of age and in suitable physical condition to tolerate the necessary investigations and major abdominal surgery. They must have an estimated survival of at least 3 years. Both sexes will be included except for exclusion of pregnant women. The study will be organized by the contractor into five separate projects. All of the various imaging methods to be investigated will be included in one of four projects. The fifth project will be concerned with collection and evaluation of data gathered by the other four projects.

Progress: During the initial nine months of the contract 30 patients with high clinical indications for probable pancreatic cancer have been entered into the study; 22 proved to have pancreatic cancer. The methods shown previously by these workers to be most useful, ultrasonography, endoscopic retrograde cholangiopancreatography, and arteriography, continue to show promising results. Although earlier radionuclide scans, using ^{75}Se-selenomethionine, were found not to be useful because of both low specificity and low sensitivity, better preliminary results have been found in the current study with ^{11}C-tryptophan. Studies with this material, produced with the local cyclotron and scanned with a special positron camera, are continuing. Preliminary work is being carried out to develop a CT ultrasound scanning system. Other work involves development of computer programs to produce a synthesized image which superimposes arteriographs and CT scan images to produce a composite image. The approach follows similar work done in cranial radiography. The various above approaches will be pursued in the second year of the study.

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 79 Funds: $223,413

CONTRACT RESEARCH SUMMARY

Title: Statistical Center for Cooperative Lung Cancer Groups

Principal Investigator: Dr. C. Ralph Buncher
Performing Organization: University of Cincinnati
City and State: Cincinnati, OH

Contract Number: NO1-CB-43868
Starting Date: 6/1/74 Expiration Date: 5/31/80

Goal: To collect and analyze data from the three collaborating clinical groups in the lung cancer screening program directed toward the detection of early lung cancer in high-risk patients to see if this screening program will reduce mortality and morbidity.

Approach: Procedures have been established and agreement has been reached concerning the common data base for this study. The data are being monitored by the Statistical Center, entered into the computer, and analyzed. Reports are being made quarterly. The Statistical Center will search the data for significant statistical findings.

Progress: The Cincinnati Central Statistical Group (CSG) has established a common data base for this three clinical center study of screening to detect lung cancer at an early enough time to reduce mortality. A reporting system for the clinical centers is in place and analyses of results to date have been provided to the NCI and participating clinical centers. Emphasis has shifted from intake of subjects to adherence and compliance with the screening program. Also efforts are now being made by the CSG to obtain meaningful results with respect to mortality differences in the screening groups at as early a time as feasible. Regular mortality conferences are held to obtain the best cause of death information for each study participant.

CSG also is cooperating with five centers in a collaborative effort to determine effectiveness of X-ray CT scanning in detection and diagnosis of brain tumors. Currently the study is nearly completed and reports are being prepared for publication. Similarly CSG is working with three centers in a collaborative study of pancreatic cancer diagnostic procedures; reports are being written.

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 79 Funds: $199,878

CONTRACT RESEARCH SUMMARY

Title: Development of Immunoglobulins Labeled with Gamma-emitting Radioisotopes

Principal Investigator:	Dr. Wayne A. Tompkins
Performing Organization:	University of Illinois
City and State:	Urbana, IL
Contract Number:	NO1-CB-64073
Starting Date:	6/30/76
Expiration Date:	6/29/79

Goal: To detect and localize, within the region of the body, cancer tissue too small to be found with currently available methods.

Approach: Detection and localization of early and/or small cancer lesions will be improved by development of immunoglobulins which are relatively specific to selected human tumor tissue components and which are labeled with suitable gamma-emitters to provide external localization of the tumor site.

During the first year, CEA and TSA (tumor-specific antigen) will be isolated from human tumors, purified, and injected into animals for antibody production. The resulting antibodies, after separation and purification, will be tested in vitro and in suitable in vivo animal models. Concurrently, development of antibody labeling techniques will be started and growth of intestinal carcinoma cells initiated in nude mice.

During the second and third years, the CEA and TSA will be produced in larger amounts, labeled, and tested in vivo in animals.

Progress: Both primary and metastatic GI-associated tumor material has been collected; this includes paracentesis fluid, liver tissue, and primary colon lesions. CEA has been isolated from this material and purified; animals have been injected with some of that material for anti-CEA immunoglobulin production. ^{51}Cr cytotoxicity studies have been made. A solid phase ^{131}I-anti-CEA binding test has been developed for quantitating CEA on the surface of HCT-8R cells. Preliminary work has been done on extractions concentrated on: continued production of CEA and anti-CEA and purification by immunoabsorbent column elution; development of an in vivo system for tumor localization of ^{125}I-anti-CEA; and further purification and evaluation of PCA-S2 as a tumor-associated antigen.

Significance to Cancer Research: This and similar studies will contribute to nuclear medicine imaging capabilities for detection and localization of cancer lesions.

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Development of Contrast Agents for Use in Clinical Ultrasonic Diagnosis

Principal Investigator: Dr. Nabil F. Maklad
Performing Organization: University of Kansas
City and State: Lawrence, KS

Contract Number: NO1-CB-84236
Starting Date: 6/26/78 Expiration Date: 6/25/80

Goal: To improve capability of ultrasound in the imaging of small tumors through the use of appropriate contrast agents.

Approach: Chemical compounds with appropriate physical properties to influence the ultrasonic signal will be identified and equipment will be assembled to test such compounds. Differential uptake of individual contrast compounds will be measured in animal tumor models. Dosage requirements for optimum ultrasonic visualization will be determined in animals and, later, in human subjects. Animal toxicity studies will be made with promising compounds. Clinical trials will be conducted in the third year of the study using the most promising contrast agent.

Progress: Speed of sound measurements have been made in a series of intravenously injectable compounds in aqueous solutions to identify potentially useful contrast agents for diagnostic ultrasound. Among the approximately twenty solutions tested those containing citrate ion were most effective. In vitro profusion studies of citrate solutions in isolated dog kidneys indicated scatter reduction and enhancement effects with 3 MHz ultrasound. These visible changes in scattering properties represent encouraging preliminary results for ultrasound contrast agents. Preliminary results also appear promising in the work to produce useful microcapsules of appropriate size (less than 6 microns) to pass through the lungs and allow ultrasound contrast imaging of selected organs such as kidney, liver, and spleen.

Significance to Cancer Research: This work will provide an indication of the potential for improvement in ultrasonic imaging of tumors through the use of contrast materials.

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 79 Funds: $199,944

CONTRACT RESEARCH SUMMARY

Title: Development of Immunoglobulins Labeled with Gamma-emitting Radioisotopes

Principal Investigator: Dr. David M. Goldenberg
Performing Organization: University of Kentucky Research Foundation
City and State: Lexington, KY

Contract Number: NO1-CB-64011
Starting Date: 6/30/76 Expiration Date: 10/31/79

Goal: To detect and localize, within the region of the body, cancer tissue too small to be found with currently available methods.

Approach: Detection and localization of early and/or small cancer lesions will be improved by development of immunoglobulins which are relatively specific to selected human tumor tissue components and which are labeled with suitable gamma-emitters to provide external localization of the tumor site.

During the first year the tumor localizing efficiency of ^{131}I-labeled anti-CEA immunoglobulins will be evaluated in animal studies. After their suitability is established by the animal studies, the materials will be tested in human subjects. The clinical testing may begin before the end of the first year and will be done first with advanced incurable cancer patients, then proceed to those with curable cancer, and finally, if the initial studies are successful, done with those having questionable cancer. Other radiolocalization systems based upon non-CEA tumor antigens will also be studied concurrently with those described above. However, since these systems are in earlier stages of development, no clinical trials will be made on them before the third year of the study.

Progress: ^{131}I-labeled anti-CEA immunoglobulin has been purified and tested for purity and suitability for human use. First clinical testing of the material was begun during the summer of 1977, and by May 1979 approximately 100 cancer patients were studied. Preliminary results are encouraging. After computer subtraction of blood pool background activity, localization of the labeled immunoglobulin could be detected, at 48 hr post-injection, in most of the cases studied. CEA-containing tumors at various body sites could be localized by external scintillation techniques. Scanning results were negative in patients without demonstrable tumors or with tumors apparently free of CEA.

Significance to Cancer Research: This and similar studies will contribute to nuclear medicine imaging capabilities for detection and localization of cancer lesions.

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Screening Technique for Blood in Stool To Detect Early Cancer of Bowel

Principal Investigator: Dr. Victor A. Gilbertsen
Performing Organization: University of Minnesota
City and State: Minneapolis, MN

Contract Number: NO1-CB-53862
Starting Date: 6/30/75 Expiration Date: 6/29/79

Goal: The goal of this project is to demonstrate a significant reduction in mortality due to colorectal cancer between the annually screened and the control group by employing the Hemoccult (R) form of the guaiac test for stool blood in combination with a diagnostic protocol for the source of bleeding.

Approach: Forty-five thousand participants between the ages of 50 and 80 years residing in the state of Minnesota are randomly allocated to three groups on the basis of age, sex, and geographic location in the state. Slides are completed after the observance of a meat-free diet with suggested high fiber content. One of the groups completes the slides once per year, another every other year, and a third group serves as the control. Participants submitting samples positive for blood receive a diagnostic examination (including a colonoscopy) to determine the cause of bleeding. All diagnostic procedures are conducted at the University of Minnesota Medical Center. All data including pathology reports on biopsy material and surgical specimens are computerized for easy retrieval. Extensive follow-up procedures are designed to retain participation of all subjects throughout the five years of testing and an additional five years of follow-up. Extensive dietary and health history data are collected from each subject via questionnaire.

Progress: The significant findings to date include an overall rate of cancer detection of about 7% of those returning positive slides; an apparent reduction in the proportion of cancers detected on successive screens for the annually screened group, similar but less definite results among the 2-year screened group; the persistence of a rate of about 25% in the detection of benign polyps across all screens. Comparative studies have begun on the diagnostic efficacy of the single-phase barium enema X-ray with the colonoscopic examination stimulated by apparent unevenness in the results of the X-ray procedure.

Follow-up of the outcome of therapy for all cases of malignancy, regardless of site, detected through the project has begun, although therapy is not a technical part of the basic project design. Investigation into the significance of rehydration versus non-rehydration of slides prior to development is continuing. The relative efficiency of the two protocols toward the detection of significant gastrointestinal pathology is unknown. A doubling of the positivity rate (2% to 4%) is persistent.

Project Officer: Louis P. Greenberg
Program: Diagnosis
Technical Review Group: Diagnosis Research Advisory Group
FY 79 Funds: $680,565

CONTRACT RESEARCH SUMMARY

Title: Development of Serum Isozyme Test for Human Cancer Patients

Principal Investigator: Dr. K. C. Tsou
Performing Organization: University of Pennsylvania
City and State: Philadelphia, PA

Contract Number: NO1-CB-64029
Starting Date: 6/30/76 Expiration Date: 12/31/79

Goal: To improve on the early diagnosis of cancer of the liver and further examine the possibilities of preventing the spread of primary cancers to other body sites with concentration on liver tissue.

Approach: (1) Further technical improvement of the 5'-nucleotide phosphodiesterase isozyme method by gel electrophoresis as a diagnostic tool; (2) further development of correlation data (biopsy, scan) and 5'-nucleotide phosphodiesterase-V for primary hepatoma; (3) further definition of possible relationships of the isozyme band V of this enzyme to liver metastasis; (4) investigation of whether there are relationships between this isozyme and other known liver markers related to hepatic cancer, especially α-fetoprotein and hepatitis B surface antigen (Australia antigen); (5) to improve the sensitivity of the test through the development of an alternate assay method, including immunoassay if an anti-enzyme antibody can be prepared with reasonable ease; (6) correlation of clinical information and other liver function tests with the results of this test; and (7) when the limitation of the test can be defined, to suggest how this test can be best used for early diagnosis.

Progress: Continuous progress has been made in using 5'-nucleotide phosphodiesterase isozyme-V (5'-NPD-V) for the detection of liver metastasis. Total number of coded samples has now reached 6,136 (from about 3,000 patients). Retrospective studies have been done mainly for patients with breast, gastrointestinal, and lung cancer. The incidences for positive 5'-NPD-V among patients with confirmed liver metastases were 103/110 (94%) for breast cancer, 172/197 (87%) for G.I. cancer, and 38/43 (88%) for lung cancer. Comparative studies with other liver function tests are currently in progress among unsuspected patients.

In addition, 5'-NPD-V were found positive in 56/63 (89%) primary hepatoma from the U.S. Of this group, 27/60 (45%) had AFP>10 ng/ml. Primary hepatoma sera were also obtained from the Far East and Africa, and they were found to be 98/127 (77%) positive for 5'-NPD-V. Similarly, AFP was found to be elevated in 68/103 (66%) in these samples. While prospective studies remain to be carried out, the results of these retrospective coded panels suggest that 5'-NPD-V is a useful predictor for both primary and secondary hepatoma.

Project Officer: Louis P. Greenberg
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 79 Funds: $80,000

CONTRACT RESEARCH SUMMARY

Title: Study of a Serum Alkaline Phosphatase Variant (FHAP) in Cancer Patients

Principal Investigator: Dr. Frank C. Larson
Performing Organization: University of Wisconsin
City and State: Madison, WI

Contract Number: NO1-CB-74173
Starting Date: 9/15/77 Expiration Date: 9/14/79

Goal: To define more clearly the possible diagnostic significance of the presence of an isoenzyme of alkaline phosphatase in serum of human cancer patients.

Approach: First, to screen a sufficient number of patients with a variety of cancers to determine whether the elevation in serum alkaline phosphatase variant (FHAP) found in preliminary studies is characteristic of all cancer types or restricted to cancers of certain organ sites and cell types. Next, to make sequential measurements in patients with cancer types which are found to be associated with FHAP to ascertain if variations in levels of serum FHAP reflect changes in cancer load following surgery, radiotherapy, or chemotherapy. Finally, to test effect of method improvement on discriminating power of the FHAP assay.

Progress: Studies continue on the relation between an isoenzyme of alkaline phosphatase and cancer. This isoenzyme is now referred to as FHAP connoting two of its distinguishing characteristics, fast migration on electrophoresis, and homoarginine inhibition. Initial reports of this relationship were based on electrophoretic separation and semiquantitative assay. Column separation, followed by a fluorometric assay, has been perfected which provides a quantitative method. This method has been used since the beginning of the current study. The three groups compared, and their values to date are:

	Mean	Median	% 2 U/L
1. Normal blood donors (N=931)	1.02	0.91	4.0
2. Patients with benign disease (N=466)	1.52	1.07	11.0
3. Patients with diagnosis of cancer (N=343)	9.20	2.31	60.0

Subgroups among the benign patients and the cancer patients are under study. Diabetes mellitus is a common cause of false positives. Cancer patients are subgrouped by organ site and by estimated tumor load. Only in the case of breast and colon cancer are numbers large enough to draw conclusions. Among these two cancer types there is a positive relationship between tumor load and the serum FHAP activity.

Project Officer: Louis P. Greenberg
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Development of Large Area Solid State Image Receptors for X-Ray Imaging

Principal Investigator: Dr. Moshe Ein-Gal
Performing Organization: Xerox Corporation
City and State: Pasadena, CA

Contract Number: NO1-CB-74211
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: To improve clinical diagnostic radiology, for use in diagnosis of early cancer, by developing an X-ray sensor superior in clinical usefulness to those currently available.

Approach: A large area solid state X-ray detector will be produced which will transform the X-ray flux into a digitally stored high quality image. The system to be developed, an X-ray selenium electronic linear scanner, will use the capability of a selenium alloy photoreceptor to create a latent image from absorbed X-rays. The latent image, an array of electric charges on the photoreceptor surface, will be detected by a microelectrometer scanning arrangement. Signals from the electrometer will be coupled with the necessary control, display, and image storage memory components. Feasibility of the imaging system components will be developed in the first year. Further design, development, and optimization of the system will be completed during the second year. Clinical evaluation of the total imaging system will be conducted during the third year.

Progress: Initial assembly and feasibility testing of the imaging system components was accomplished during the first year as scheduled. During the second year, additional key component systems are being procured and integrated into the system. After additional development and optimization during summer 1979 the system will be ready for clinical feasibility testing in late fall 1979.

Significance to Cancer Research: This system will allow digital storage, transmission, retrieval, and selection of imaging information.

Project Officer: R. Q. Blackwell, Ph.D.
Program: Diagnosis
Technical Review Group: Diagnostic Research Advisory Group
FY 79 Funds: $360,000 estimated

CYTOLOGY AUTOMATION CONTRACT PROGRAM

CYTOLOGY AUTOMATION CONTRACT PROGRAM

The Committee on Cytology Automation was established in July 1972 to advise the Director, Division of Cancer Biology and Diagnosis, National Cancer Institute, on all matters pertaining to Cytology Automation. Specifically, the Committee (1) serves as advisors to National Cancer Institute on the state of the art in scientific and technical areas which bear on automation, and (2) advises the National Cancer Institute on plans and implementation in operational terms for National Cancer Institute's contractual and grant support of developing automation in cytopathology.

The Committee determined that six areas are within the mission of the Committee:

1. Specimen Collection
2. Specimen Preparation
3. Sensing-screening Systems
4. Decision-diagnostic Systems
5. Specimen Sources
6. System Evaluation

At the present time, work in several very important areas of research has been accomplished. In area 1, specimen collection, Dr. Marluce Bibbo and collaborators at the University of Chicago determined in an extensive study that the number of single abnormal cells present in gynecologic specimens is adequate for screening purposes in essentially all cases of pre-malignant and malignant lesions. Thus, only relatively gentle disaggregation procedures are necessary in preparing clinical specimens for automated analysis.

In area 2, specimen preparation, several contractors have completed a thorough examination of mechanical and enzymatic dispersal procedures to increase the yield of single intact cells. The results of this work indicate that relatively simple mechanical dispersal techniques, such as pressure-controlled syringing and ultrasonic treatment, produce adequate numbers of morphologically and cytochemically intact cells for instrumental analysis.

In areas 3, 4, and 6, development and evaluation of integrated screening systems, several automated analytic systems have been identified as promising potential designs for automated clinical prescreening systems. Evaluation and development of these systems is proceeding to: (1) ensure that they reflect a sound biologic basis for identification of pre-malignant and malignant squamous cell lesions of the gynecologic tract; and (2) evaluate their performance characteristics, especially preliminary analysis of sensitivity and specificity.

A flow microfluorometric system developed by this program for screening has been documented in a double-blind clinical trial to perform at a level of false-negative errors comparable to that reported for manual screening.

Work is continuing on the identification of quantitative descriptors of pre-malignant and malignant lesions, especially cytochemical and immunological markers which will increase the potential sensitivity and specificity of

currently employed sensor systems in screening clinical material. In addition, research is in progress to test a module sorter to separate cells based on individual cell parametric measurements, assuring a correlation of individual separated cells with specific quantitative measurements in flow systems. The module will be interfaced to a multiparameter flow sorter. Research is developing and testing single cell classification algorithms employing the current state of the art in digital image processing, scene segmentation, and image acquisition hardware. An integral system and algorithms are being evaluated to determine the classification performance for screening squamous epithelial cells. work is in progress in the comparison of instrumental or system classification and microscopic "true classification" using standard morphologic criteria. The data will have a direct bearing on the analysis of the performance of any automated systems.

CONTRACT RESEARCH SUMMARY

Title: Development of New Methods of Single Cell Separation

Principal Investigator: E. Robert Schildkraut
Performing Organization: Block Engineering, Inc.
City and State: Cambridge, MA

Contract Number: N01-CB-74095
Starting Date: 8/17/77 Expiration Date: 8/16/80

Goal: Development and testing of a single cell separator that will reliably associate individual separated cells with specific quantitative measurements.

Approach: The contractor shall develop and analyze techniques for deposition, fixation, staining, and scribing system for gynecologic cells on film. The speed and fluid volumes will be compatible with the output of a flow microfluorometric system. The design of this scribing system will not be such as to preclude its adaptation as a module to presently available FMF systems other than the contractor's. The contractor shall also develop and analyze a fiducial system which will allow reliable unique recall and identification of individual cells.

Progress: A tape-based single cell collection system has been constructed and tested. Its operation is acceptable and during the current year it will be interfaced to a research model flow microfluorometer supported by the Cytology Automation Program for extensive testing of gynecologic specimens.

Significance to Cancer Research: The development of a reliable clinical screening system depends on the identification and assurance that "abnormal" quantitative signals are associated with abnormal cells.

Project Officers: Bill Bunnag, Ph.D., Chester J. Herman, M.D., Ph.D.
Program: Cytology Automation
Technical Review Group: Committee on Cytology Automation
FY 79 Funds: $43,316

CONTRACT RESEARCH SUMMARY

Title: New Stains and Other Optical Markers Useful for Gynecologic Specimens

Principal Investigator: Dr. Brian H. Mayall
Performing Organization: DOE--Lawrence Livermore Laboratory
City and State: Livermore, CA

Contract Number: Y01-CB-40300
Starting Date: 6/1/74 Expiration Date: 6/29/80

Goal: Development and preliminary clinical testing of stains useful in differentiating normal from non-normal cytopathological material.

Approach: The contractor will continue to implement and evaluate the dual laser sorter. The development and evaluation of new markers for automation of clinical cytology will continue. Evaluation will be carried out on the dual laser sorter. Computer controlled sorting will be completed and interfaced to the dual laser system. Subpopulation of clinical cytology specimens from normal to premalignant and malignant cases will be identified using new staining and single and multiparameter analysis techniques. These subpopulations will be sorted and analyzed after morphologic characterization.

Progress: Protocols have been developed and implemented for cell suspension and centrifugal cytology for routine cytodiagnosis as well as for experimental procedures. The use of dual staining and analysis with the dual beam sorter has shown an identifiable subpopulation in gynecologic cytology specimens which has altered chromatin structure. The computer controlled sorting hardware and software are being tested.

Significance to Cancer Research: Development and evaluation of techniques to rapidly screen cell populations for premalignant and malignant cells.

Project Officers: Mary C. Habbersett, Bill Bunnag, Ph.D.
Program: Cytology Automation
Technical Review Group: Committee on Cytology Automation
FY 79 Funds: $346,426

CONTRACT RESEARCH SUMMARY

Title: Application of Flow System Methods of Cell Analysis and Sorting to Cancer Screening

Principal Investigator: Dr. Paul Mullaney
Performing Organization: DOE--Los Alamos Scientific Laboratory
City and State: Los Alamos, NM

Contract Number: YO1-CB-60311
Starting Date: 4/1/71 Expiration Date: 5/31/80

Goal: Automation of clinical cytology screening and diagnosis.

Approach: The contractor shall (1) continue and conclude the development and evaluation of the parabolic flow chamber, and (2) continue and conclude fabrication and evaluation of the two laser cell analysis and sorting system including measurements of cells stained with two or more fluorochromes, two-color light scattering measurements and Coulter volume, and (3) develop protocols for new fluorochromes using model cell systems and clinical material.

Progress: The new parabolic flow chamber has been machined and is undergoing testing. The overall collection efficiency is about 75%. The fabrication of the dual laser sorter has been completed. Newly designed signal processing hardware is under evaluation. Several fluorochrome combinations have been tried using model cell systems and clinical gynecologic material.

Significance to Cancer Research: The use of a very rapid zero-resolution automated screening system in clinical cytodiagnosis will allow greater availability of these services to a much larger population with improved diagnostic accuracy.

Project Officers: Mary C. Habbersett, Bill Bunnag, Ph.D.
Program: Cytology Automation
Technical Review Group: Committee on Cytology Automation
FY 79 Funds: $249,300

CONTRACT RESEARCH SUMMARY

Title: Vaginal-Cervical Sample Sources

Principal Investigator: Dr. Gregorio Delgado
Performing Organization: Georgetown University Hospital
City and State: Washington, DC

Contract Number: NO1-CB-70482
Starting Date: 6/30/76 Expiration Date: 6/29/79

Goal: To supply NCI and contractors with samples to evaluate their experiments and instrumentation.

Approach: The contractor shall deliver to the Laboratory of Pathology 990 gynecologic cytopathology samples obtained according to a protocol supplied by NCI. These samples will range in diagnoses from normal to squamous cell carcinoma and adenocarcinoma. They will be used to evaluate instrumentation and methods of sample preparation and staining, both at NCI and at other contracting institutions within this program.

Progress: The contractor has delivered to NCI gynecologic cell samples ranging from normal to invasive squamous cell carcinoma. These samples are used in sample preparation studies as well as for evaluating NCI's multiparameter cell sorter. A number of these samples have been sent to other contractors for use in their studies.

Significance to Cancer Research: The accessibility of gynecologic cytopathology samples of all types of abnormality will expedite the evaluation and experimentation in the program of automated cytology.

Project Officer: Mary C. Habbersett
Program: Cytology Automation
Technical Review Group: Committee on Cytology Automation
FY 79 Funds: $10,000

CONTRACT RESEARCH SUMMARY

Title: Immunologic Markers Applicable to Cytology Automation

Principal Investigator: Dr. Laure Aurelian
Performing Organization: Johns Hopkins University
City and State: Baltimore, MD

Contract Number: NO1-CB-74170
Starting Date: 7/18/77 Expiration Date: 7/17/80

Goal: Qualitative and/or quantitative antigenic changes in premalignant and malignant cells will allow discrimination from normal cells.

Approach: The contractor shall isolate HSV antigens: (i) Total viral antigens, (ii) AG-e, and (iii) fractions of AG-e, and prepare corresponding purified antisera to test sensitivity and specificity of these antisera to human gynecologic materials. Immunological and biochemical characterization of the antigens will be done.

Progress: The preparation of antigens from HSV infected cells and the purification of the antigens have proceeded as scheduled. Testing by indirect immunofluorescent staining appears promising. A double-blind study using the antisera already prepared is being designed for implementation this year. The study will include specimens from patients with a wide variety of diagnoses ranging from normal to invasive cancer and will include a variety of inflammatory atypias including herpetic. The study will compare immunofluorescent marking of specimens on both a specimen-by-specimen and cell-by-cell basis with routine manual screening and microscopic examination.

Significance to Cancer Research: The development of new markers will improve the prospects for more rapid automation of clinical cytopathologic prescreening.

Project Officers: Bill Bunnag, Ph.D., Chester J. Herman, M.D., Ph.D.
Program: Cytology Automation
Technical Review Group: Committee on Cytology Automation
FY 79 Funds: $90,658

CONTRACT RESEARCH SUMMARY

Title: Application of Digital Image Processing Techniques to Cytology Automation

Principal Investigator: Dr. Kenneth Castleman
Performing Organization: JPL--California Institute of Technology
City and State: Pasadena, CA

Contract Number: YO1-CB-70314
Starting Date: 9/30/77 Expiration Date: 9/29/80

Goal: Development of optimal algorithms for cell classification applicable to automated digital image processing system.

Approach: The contractor shall determine whether or not current state of the art in pattern recognition is adequate to support the development of an economically viable cytological screening instrument based on single cell classification. Specimen preparation protocol will be optimized so as to produce acceptable single cells for digitization. A large number of digitized images will be accumulated in the library for subsequent feature extraction program to produce a feature data base. A series of classification experiments will be run on the feature data base to select the subsets of cell measurements that yield the best overall performance.

Progress: Specimen preparation protocol has been completed. Digitized cell images were acquired and classification algorithms developed. The results of this portion of the study indicate that the cell classification algorithms chosen can perform at an error rate consistently better than that achieved by routine manual screening. The second phase of the study has been initiated. This phase will evaluate the performance of the cell classification algorithms on cells selected and segmented without human interaction.

Significance to Cancer Research: Single cell classification algorithms are the key to success in any automated digital image processing system applied to cytology automation.

Project Officers: Bill Bunnag, Ph.D., Chester J. Herman, M.D., Ph.D.
Program: Cytology Automation
Technical Review Group: Committee on Cytology Automation
FY 79 Funds: $252,926

CONTRACT RESEARCH SUMMARY

Title: Development of Hematoxylin Substitutes for Staining of Tissues and Exfoliated Cells

Principal Investigator: Dr. Ralph D. Lillie
Performing Organization: Louisiana State University
City and State: Baton Rouge, LA

Contract Number: NO1-CB-43912
Starting Date: 1/11/74 Expiration Date: 1/10/79

Goal: Development of hematoxylin substitutes for diagnostic staining of tissues and exfoliative cells.

Approach: (1) Synthesize various classes of mordant dyes or obtain same from either specific manufacturers or from the archives of the Biological Stain Commission. (2) Perform studies on various tissues with combinations of dyes obtained to achieve staining in accordance with criteria satisfactory to the project officer and to the Biological Stain Commission's certification criteria. (3) Procure samples of possibly suitable mordant dyes offering blue or black mordant colors which may be suitable to replace hematoxylin in one or more of its histological staining uses. (4) Subject such samples to the usual tests applied by the Biological Stain Commission for the certification of hematoxylin. (5) Apply favorable test results to routine surgical diagnostic material, in Papanicolaou smear diagnosis, and in other staining procedures applicable to the differential diagnosis of various cancers, evaluating results in each case in comparison of previously standard hematoxylin procedures and with the best of the previously proposed substitutes.

Progress: Synthesis and testing are on schedule as outlined above.

Significance to Cancer Research: Stain development is essential to the cytological diagnosis of cancer.

Project Officer: Mearl F. Stanton, M.D.
Program: Cytology Automation
Technical Review Group: Ad Hoc Committee Laboratory of Pathology
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Immunologic Markers Applicable to Cytology Automation

Principal Investigator: Dr. John J. Docherty
Performing Organization: Pennsylvania State University
City and State: University Park, PA

Contract Number: NO1-CB-74171
Starting Date: 7/18/77 Expiration Date: 7/17/79

Goal: Qualitative and/or quantitative antigenic changes in premalignant and malignant cells will allow discrimination from normal cells.

Approach: The contractor shall isolate "tumor antigen" extracted from HSV-1 and HSV-2 transformed cells, and prepare corresponding purified antisera for labeling with peroxidase and fluorescein isothiocyanate and testing on gynecologic exfoliated cervical cells and biopsy specimens. Cell by cell analysis will be done to assess the sensitivity and specificity of the assay. Techniques for immunofluorescent staining of exfoliated cells in suspension will be developed for use with flow microfluorometric cell sorter.

Progress: Exfoliated cells are being used to test the sensitivity and specificity of the isolated "tumor antigen." The cells are subsequently destained and restained by the "Pap" procedure for diagnosis. Results indicate that the specificity and sensitivity of these markers are good in patients with known premalignant and malignant lesions. The use of these data as prognostic and therapeutic indicators in treated patients is being pursued utilizing grant funding.

Significance to Cancer Research: The development of new markers will improve the prospects for more rapid automation of clinical cytopathologic prescreening.

Project Officers: Bill Bunnag, Ph.D., Chester J. Herman, M.D., Ph.D.
Program: Cytology Automation
Technical Review Group: Committee on Cytology Automation
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Application of Digital Image Processing Techniques to Cytology Automation

Principal Investigator: Dr. James W. Bacus
Performing Organization: Rush-Presbyterian-St. Luke's Medical Center
City and State: Chicago, IL

Contract Number: NO1-CB-74190
Starting Date: 9/30/77 Expiration Date: 9/29/81

Goal: Development of optimal algorithms for cell classification applicable to automated digital image processing system.

Approach: The contractor shall conduct a comprehensive study to determine single cell vs. specimen classification accuracies for image processing algorithms and for cytotechnologist. This will determine how well the machine algorithms are performing. Five experimental tasks will be done: (1) sample acquisition, (2) cell acquisition, (3) observer recognition, (4) cell classification algorithms development, and (5) analysis and evaluation of results.

Progress: Patient selection and sample acquisition are close to completion. Fabrication of the cell acquisition system is well under way. Software is being written for the various aspects of the cell acquisition protocol. Preliminary work leading up to the beginning of the cell acquisition experiment has been completed. Programming of cell recognition algorithms continues with emphasis on scene segmentation techniques.

Significance to Cancer Research: Single cell classification algorithms are the key to success in any automated digital image processing system applied to cytology automation.

Project Officers: Mary C. Habbersett, Bill Bunnag, Ph.D.
Program: Cytology Automation
Technical Review Group: Committee on Cytology Automation
FY 79 Funds: $382,181

CONTRACT RESEARCH SUMMARY

Title: Methods for Obtaining Monodisperse Preparations of Cytology Specimens

Principal Investigator: Dr. Walter E. Tolles
Performing Organization: State University of New York
City and State: Brooklyn, NY

Contract Number: NO1-CB-43955
Starting Date: 6/1/74 Expiration Date: 5/31/79

Goal: To obtain a monodisperse cell sample from gynecologic cytologic specimens for use in flow-through and image processing systems being developed for cytology automation, and to supply NCI and other contractors with treated and untreated gynecologic samples for use in the evaluation of sample preparation and instrumentation.

Approach: The contractor shall deliver to the Laboratory of Pathology or other contractors at least 12 specimens per week ranging in classification from normal to invasive carcinoma. These specimens will be either untreated or disaggregated by ultrasound.

Progress: The gynecologic cytology samples which have been delivered to NCI have been adequate in diagnostic cellular material. The samples sent have been either untreated in suspension or enriched in suspension or on slides. A portion of these samples has been sent to other contractors for use in their studies.

Project Officer: Mary C. Habbersett
Program: Cytology Automation
Technical Review Group: Committee on Cytology Automation
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Vaginal and Cervical Cell Sample Sources for Cytology Automation

Principal Investigator: Dr. Parviz Hanjani
Performing Organization: Temple University
City and State: Philadelphia, PA

Contract Number: 263-77-C-0513
Starting Date: 6/20/74 Expiration Date: 6/30/79

Goal: To supply NCI and contractors with samples to evaluate their experiments and instrumentation.

Approach: The contractor shall deliver to the Laboratory of Pathology 600 gynecologic cytopathology samples a year obtained according to a protocol supplied by NCI. These samples will range in diagnoses from normal to squamous cell carcinoma and adenocarcinoma. They will be used to evaluate the multiparameter cell sorter and methods of staining and cell dispersal being investigated for eventual use in a fully automated clinical instrument.

Progress: The contractor has delivered to NCI gynecologic cell samples ranging in diagnosis from normal through invasive squamous cell carcinoma. These samples are used in sample preparation studies as well as for evaluating NCI's multiparameter cell sorter. A number of these samples have been sent to other contractors for use in their studies.

Significance to Cancer Research: The accessibility of gynecologic cytopathology samples of all types of abnormality will expedite the evaluation and experimentation in the program of automated cytology.

Project Officer: Mary C. Habbersett
Program: Cytology Automation
Technical Review Group: Committee on Cytology Automation
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Image Processing for Development of Automated Cell Recognition System

Principal Investigator: Dr. George L. Wied
Performing Organization: University of Chicago
City and State: Chicago, IL

Contract Number: NO1-CB-33873
Starting Date: 5/31/73 Expiration Date: 4/30/81

Goal: Automation of clinical cytologic screening and diagnosis, focusing on definition of information requirements and criteria for high throughput, high resolution processing-based decisionmaking.

Approach: The contractor shall evaluate information requirements for characterization and recognition of normal, premalignant, and malignant cells from the human female genital tract by means of digital image processing.

Progress: Digitized cell images have been compiled. These data are stored on tapes and will be made available to other contractors for further analyses. These cells are from the various cell types encountered in the exfoliated cell specimens from the female genital tract. Image editing system is operational. Sorted cleaned cell images are available for other contractors to analyze with their own algorithms. Further details on scanned cells are being done using 3-color scanner. A new computer program has been written to evaluate the new information. The multi-spectral band scanner has enriched the data base for cell image processing. The new color features enhance the classification of metaplasias, dysplasias, and malignant cases. Feature extraction for chromatin distribution patterns has been implemented. High-resolution data acquisition module and cell detection module hardware are being implemented. An integrated system is being tested using the best and optimal algorithms to determine the classification performance of the system to classify cells as normal and non-normal.

Significance to Cancer Research: A rapid, high-resolution, multi-spectral digital scanner for clinical gynecologic screening and diagnosis will promote better health care delivery and therapy monitoring for the populations at large and at high risk.

Project Officers: Bill Bunnag, Ph.D., Chester J. Herman, M.D., Ph.D.
Program: Cytology Automation
Technical Review Group: Committee on Cytology Automation
FY 79 Funds: $279,616

CONTRACT RESEARCH SUMMARY

Title: Immunologic Markers Applicable to Cytology Automation

Principal Investigator: Dr. Harold Haines
Performing Organization: University of Miami
City and State: Miami, FL

Contract Number: NO1-CB-74096
Starting Date: 5/13/77 Expiration Date: 5/12/79

Goal: Qualitative and/or quantitative antigenic changes in premalignant and malignant cells will allow discrimination from normal cells.

Approach: Contractor shall isolate and purify cervical carcinoma antigen (CCA). Appropriate antisera to CCA will be prepared and cell-by-cell analysis using indirect immunofluorescent technique and restaining by Pap staining procedure will be done. Exfoliated cells, tissue imprints, and tissue sections will be used. Technique for immunofluorescent staining of exfoliated cells in suspension will be developed for use in flow microfluorometric cell sorter.

Progress: Crude and purified CCA have been accumulated and corresponding antisera obtained. Exfoliated gynecologic specimens have been tested. Purification and characterization of the antigen and additional cell-by-cell analysis have revealed a complex association between CCA and premalignant, malignant, and viral-induced changes in cervical squamous epithelium. The contract has been successfully completed. Further investigation of the fundamental cell biological mechanisms responsible for the observed phenomena is being pursued utilizing grant support.

Significance to Cancer Research: The development of new markers will improve the prospects for more rapid automation of clinical cytopathologic prescreening.

Project Officers: Bill Bunnag, Ph.D., Chester J. Herman, M.D., Ph.D.
Program: Cytology Automation
Technical Review Group: Committee on Cytology Automation
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Slit-Scan Technique as Cancer Prescreening Automated System in Cytology

Principal Investigator: Dr. Leon L. Wheeless, Jr.
Performing Organization: University of Rochester
City and State: Rochester, NY

Contract Number: NO1-CB-33862
Starting Date: 3/19/73 Expiration Date: 3/18/82

Goal: Automation of clinical cytologic screening and diagnosis.

Approach: The contractor will evaluate the performance characteristics of the X-Y-Z Slit-Scan Flow System. A benchmark study of the X-Y-Z system will be performed to (1) determine system characteristics including rate and causes of remaining false alarms and (2) document true alarm rates for abnormal specimens. Correlation studies will continue and studies on second stage processing techniques will begin. The Block Engineering CSAR will be interfaced to the flow system and be evaluated for potential use in second stage screening.

Progress: The X-Y-Z Slit-Scan Flow System has been fabricated and tested. The benchmark study will begin shortly; the evaluation of the performance characteristics has begun. Sample preparation protocols continue to be evaluated and improved.

Significance to Cancer Research: The development of a flow-through screening system for cervical cytology will allow greater accuracy, speed, and availability in screening and diagnosis of cancerous and precancerous lesions.

Project Officers: Mary C. Habbersett, Bill Bunnag, Ph.D.
Program: Cytology Automation
Technical Review Group: Committee on Cytology Automation
FY 79 Funds: $385,235

BREAST CANCER TASK FORCE PROGRAM

CONTRACT RESEARCH SUMMARY

Title: Biological Markers in Breast Cancer: Patient Resource

Principal Investigator: Dr. Harold E. Bowman
Performing Organization: Butterworth Hospital
City and State: Grand Rapids, MI

Contract Number: NO1-CB-74213
Starting Date: 9/15/77 Expiration Date: 9/14/80

Goal: To develop a Breast Cancer Task Force specimen resource for blood from breast cancer patients, benign disease patients, and controls to be used in a search for and verification of new breast cancer markers.

Approach: Thirty milliliters of blood will be collected from breast cancer patients who are scheduled to undergo biopsy and/or primary surgery for breast lesions prior to surgery. Another specimen will be collected, when feasible, 5-10 days postmastectomy from the same patient. Serum specimens will be stored at -70°C, then shipped to an NCI-designated blood bank facility with appropriate clinical data.

Progress: The program has been presented to the surgery sections in each participating hospital. Surgeons who perform 95% of all breast biopsies in the participating hospitals have signed letters of agreement allowing their patients to enter directly into the study. Since the inception of the program, 719 patients have been entered into the study; 157 of these patients have been found to have malignant breast disease; and 8,974 vials containing serum specimens have been shipped to the central storage facilities at Mayo Clinic. Of these vials, only about 2% have been found to be unsatisfactory for research purposes.

Project Officers: Ihor J. Masnyk, Ph.D., Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $28,500

CONTRACT RESEARCH SUMMARY

Title: Biologic Markers in Breast Cancer: Patient Resource

Principal Investigator:	Dr. Ned D. Rodes
Performing Organization:	Cancer Research Center
City and State:	Columbia, MO

Contract Number: NO1-CB-74138
Starting Date: 9/1/77 Expiration Date: 8/31/80

Goal: To serve as a Breast Cancer Task Force specimen resource for blood from breast cancer patients and controls to be used in a search for and verification of new breast cancer markers.

Approach: Thirty milliliters of blood will be collected from all Breast Cancer Detection Demonstration Projects screenees (approximately 600 per month) who have signed appropriate consent forms. One 30 ml postoperative specimen will also be collected from postmastectomy cases within the first month. Another specimen will be collected from previous donors at one year anniversary. No special requirements (diet, liquid) are to be placed on the volunteers. All blood will be stored at -70°C, then shipped to an NCI-designated blood bank facility with appropriate clinical data. This project will consist of a two-year collection and a three-year follow-up period.

Progress: In a nine-month period of collection (from December 9, 1977 to September 30, 1978) 3,281 individual samples were fully processed. In the subsequent six-month period, September 27, 1978 to March 19, 1979, we have had in the project, 110 days of active collection with 2,292 persons participating and fully processing 2,211. The overall average collection per subject has increased to 10.6 ml of finished double centrifuged serum or expressed in other factors, 56,295 individual samples of 1.1 ml each. Our average acceptability for research through shipment number 32, ending February 20, 1979, has been 97.27 percent.

A new project coordinator has been recruited and has begun on April 1, 1979, at a 20 percent level of time commitment and commensurate salary.

Project Officers: Ihor J. Masnyk, Ph.D., Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $105,150

CONTRACT RESEARCH SUMMARY

Title: Biologic Characterization of "Premalignant" Human Mammary Epithelial Hyperplasias

Principal Investigator: Dr. Kenneth Scott McCarty, Jr.
Performing Organization: Duke University
City and State: Durham, NC

Contract Number: NO1-CB-84223
Starting Date: 8/1/78 Expiration Date: 7/31/80

Goal: To identify and characterize potentially "premalignant" epithelial hyperplasias of the human mammary gland utilizing biochemical, morphologic, and immunologic techniques in a search for markers of malignant potential.

Approach: Material will be evaluated from 200 subcutaneous mastectomy specimens in which the mastectomy was performed for indications other than suspected or overt malignancy (50 cases selected from cosmetic surgery as "normals"), and 150 biopsies with benign lesions (biopsy performed for suspected malignancy), and 150 samples obtained from non-carcinomatous areas of mastectomy specimens in which mastectomy was performed for overt malignancy. These will be compared to carcinomatous areas of the 150 breast cancer specimens. Lesions are classified morphologically by subgross, light, and electron microscopical techniques as appropriate. The presence of steroid receptors, cyst fluid derived estrogen-progesterone binding protein, and endogenous peroxidase are compared to the presence or absence of K-casein, and CEA. Immunologic characterizations are approached by the assessment of IgG, IgA, and IgM localization with characterization of eluted immunoglobulin in selected cases. The expression of cell surface changes as manifested by the presence or absence of ABO blood group antigens and/or the unmasking of component antigens will be assessed by immunohistology. The expression of MMTV-related antigens will be studied. Comparison of these various parameters are made to thymidine labeling indices (TLI) as a reflection of "S phase" activity in specific lesions. Selected epithelial hyperplasias are studied in organ and tissue culture.

Progress: A statistically significant association of the simultaneous presence of certain intraductal epithelial hyperplasias and carcinoma has been shown. Apocrine and adenosquamous differentiation in mammary carcinomas has been characterized ultrastructurally. Comparison of immunoglobulin localization patterns and ABO blood group antigen to alterations in the epithelial stromal junction (ESJ) lends support to the importance of the ESJ in mammary dysplasias. The localization of immunoglobulin G in a pericellular pattern has been observed in dysplastic epithelial hyperplastic lesions while apical IgA localization has characterized the benign lesions studied.

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $74,100

CONTRACT RESEARCH SUMMARY

Title: To Code Additional Data for Breast Cancer Marker Program

Principal Investigator: Dr. Raymond Fink
Performing Organization: Health Insurance Plan of Greater New York
City and State: New York, NY

Contract Number: NO1-CP-43278
Starting Date: 12/1/77 Expiration Date: 11/30/78

Goal: To provide clinical backup for serum specimens collected in the HIP Mammography Study.

Approach: The abstractors will search records of the identified blood donors and report on study forms.

Progress: The blood samples have been inventoried, the ballast (unusables) has been discarded, and a list of outstanding cases has been prepared. Data abstracting is in progress.

Project Officer: Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Isoproteins in Normal, Benign, and Malignant Breast Tissues

Principal Investigator: Dr. Doris Balinsky
Performing Organization: Iowa State University
City and State: Ames, IA

Contract Number: NO1-CB-84222
Starting Date: 8/1/78 Expiration Date: 7/31/80

Goal: To detect, quantitate, and characterize various isozymes or isoproteins in normal, benign, and neoplastic breast tissue in an attempt to identify "tumor markers" which may have significance in the early detection of breast cancer or identification of patients with occult dissemination after local treatment.

Approach: A number of enzymes including lactate dehydrogenase, malate dehydrogenase, G6PD, hexokinase, pyruvate kinase, phosphofructokinase, aldolase, alkaline and acid phosphatases, esterases, etc. will be assayed in human normal, benign, malignant, and metastatic breast tissues (50 each annually). Patterns of several of these enzymes of interest will be examined in these same tissues. Subsequently these patterns will be examined as well in the sera of normal and breast cancer patients. If the altered isoproteins are isoenzymes, they will be purified from both normal and neoplastic breast tissues and characterized. If the isoprotein is non-enzymic, it will be purified and antibody made to it for use in quantitative radioimmunoassay. These data will be correlated with data on estrogen and progesterone receptors on the same material and with histopathologic and clinical data. These studies could possibly give a lead to the altered metabolic status of the tumor.

Progress: Part of the first 6-month period was spent in setting up and equipping a laboratory in order to fulfill the contract, and in hiring and training a technician. Using a few preliminary specimens, spectrophotometric assays and electrophoretic separations were developed and optimized for lactate, malate, isocitrate and glucose 6-phosphate dehydrogenases, hexo-, pyruvate and create kinases and alkaline phosphatase. The stabilities of some of the enzymes in the frozen tissue and stored homogenates have been examined. Most of the ones examined were stable in liquid nitrogen, but less so at -20°C. Electrophoresis on starch gel, polyacrylamide gel, and cellulose acetate was tried to determine the best medium for separation of the various isozymes. In general, cellulose acetate gave good and quick separations and the stained bands could be readily scanned in a Beckman densitometer. In addition to staining with formazan stains, which give a blue color against a white background, fluorescent stains requiring contact photography under ultraviolet light have been examined, especially for pyruvate kinase and alkaline phosphatase.

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $66,400

CONTRACT RESEARCH SUMMARY

Title: The Use of Ultrasound in the Diagnosis of Breast Cancer

Principal Investigator: Dr. Barry Goldberg
Performing Organization: Jefferson Medical College
City and State: Philadelphia, PA

Contract Number: NO1-CB-84273
Starting Date: 5/1/78 Expiration Date: 4/30/80

Goal: To develop characteristic ultrasound patterns of benign and malignant breast pathology in order to evaluate the efficacy of high resolution ultrasonic equipment in the detection of early breast cancers.

Approach: Two different ultrasonic machines, the Octoson B-scan and the Johnson and Johnson real-time scanner, will be clinically evaluated. About 100-150 patients coming to surgery for suspected breast carcinoma will undergo a preoperative X-ray mammogram, physical examination, and ultrasonic examination. There will be repeat examinations of the excised breasts. In addition, 8-10 biopsy cases/week recommended for biopsy or aspiration of suspected cysts will be similarly examined. Five hundred asymptomatic patients will also be enrolled per year. Ultrasonic patterns will be correlated with histopathology and X-ray mammograms in both in vitro and intact breast tissues. A secondary objective will be to advance the state of art of detection of breast cancer by incorporating ultrasonic wave form signal analysis and acoustic signaling techniques in a computer learning procedure under subcontract.

Progress: The Octoson ultrasound machine has been modified to obtain wave forms. The techniques of analysis of this information have been established. The techniques of breast evaluation have evolved with approaches developed that ensure the visualization not only of the main portion of the breast, but also the axillary region as well. A system has been developed that enables us to accurately localize masses or areas within the breast so that they can be compared to the surgical specimens. The correlation program with pathology and X-ray mammograms has been developed. Four hundred ninety patients have been examined to date, including those with known masses as well as those in whom masses were not suspected. These breasts were evaluated according to the research proposal for presence of mass as well as analysis of breast patterns. The preliminary results show a population sensitivity of 80% and specificity of 65% for breast cancer using ultrasound evaluation. In addition, the true positive rate for ultrasound in the biopsy cases is 61% and the true negative rate is 92% (152 biopsies). Perhaps most encouraging is that ultrasound was able to detect five of the six minimal cancers present within the population.

Project Officers: Bernice T. Radovich, Ph.D., Thomas Shawker, M.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $232,000

CONTRACT RESEARCH SUMMARY

Title: Immunologic Cross-Reactions Between Breast Cancer and Mammary Tumor Virus

Principal Investigator: Dr. Grace B. Cannon
Performing Organization: Litton Bionetics, Inc.
City and State: Kensington, MD

Contract Number: NO1-CB-74087
Starting Date: 7/1/77 Expiration Date: 6/30/80

Goal: To perform the leukocyte migration inhibition (LMI) assay for the detection of cell-mediated immunity (CMI) against mouse mammary tumor virus (MMTV) and associated glycoprotein (gp-52) antigens in order to ascertain the usefulness of the LMI assay for the early diagnosis, monitoring, and prognostic predictability of patients with breast cancer.

Approach: Peripheral blood leukocytes from breast cancer patients (prior to biopsy and post-mastectomy), benign disease patients (pre- and post-biopsy), patients with other types of cancers, patients with gynecological disease, and healthy female controls will be tested under code in the LMI with MMTV and gp-52.

Progress: In the direct leukocyte migration inhibition assay, the proportion of preoperative breast cancer patients (6/35, 17%) reactive to MMTV was similar to the collective proportion of preoperative benign breast disease patients (13/136, 10%) and to the proportion of preoperative gynecological patients (3/42, 7%). The proportion of preoperative reactivity to gp-52 was slightly higher in the breast cancer patients (9/31, 29%) than in the benign patients (20/116, 17%) and the gynecological patients (4/37, 11%). Certain categories of benign breast disease (intraductal papilloma, duct ectasia, sclerosing adenosis, fibro fatty tissue) were more reactive than others (fibrocystic mastopathy and fibroadenoma). Preliminary results with the indirect migration inhibition assay were more applicable to diagnosis in that a higher proportion of preoperative breast cancer patients (4/7, 57%) than preoperative benign breast disease (7/30, 23%) and gynecological patients (1/12, 8%) were reactive. In the direct assay, postoperative reactivity to MMTV was higher in the Stage I patients (24/43, 56%) and the Stage II patients (7/25, 28%) than the benign breast disease patients (7/64, 11%). Too few patients have relapsed clinically to make a meaningful interpretation of the applicability of this assay to prognosis in this study.

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $142,363

CONTRACT RESEARCH SUMMARY

Title: Breast Diagnosis: Quantitative Imaging by Ultrasound

Principal Investigator: Dr. James F. Greenleaf
Performing Organization: Mayo Foundation
City and State: Rochester, MN

Contract Number: N01-CB-64041
Starting Date: 6/30/76 Expiration Date: 12/31/78

Goal: To determine if images representing quantitative distributions of basic mechanical tissue properties or acoustic parameters of tissue such as velocity, attenuation, reflection, impedance, and scattering can be useful in breast cancer diagnosis and of more value than images representing tissue interfaces and geometries such as B-scans.

Approach: A transverse radial scanning system has been designed and constructed which acquires ultrasound data from excised tissues and patients in several modes including echoes for compound B-scans, time-of-flight, and attenuation data for mathematical reconstruction. The ability of acoustic tissue characterization to detect and distinguish excised normal and diseased tissues is being studied by comparing results from visual evaluation and computer-aided feature extraction and pattern recognition of the various acoustic images with computer encoded histologic characteristics obtained from microscopic evaluation of tissue sections removed from the intact organ. Initial studies have begun of normal and diseased breasts in vivo in selected patients and the resulting acoustic images are being compared to histology obtained from breasts of those women who undergo mastectomy.

Progress: Signal preprocessing systems and scanner control electronics have been designed and constructed. Software programs for data acquisition, analysis, and display have been written and documented. Tomograms of acoustic speed of sound have been obtained from coronal planes through breasts obtained postmortem and in vivo. Two-dimensional distributions of acoustic speed are calculated using computer-assisted tomography. Six hundred thirty-one regions from 73 breasts on which acoustic speed and attenuation tomograms were obtained in vivo have been compared to histology of the breasts after mastectomy or biopsy. Pattern recognition methods were applied to in vivo data from 88 regions resulting in a correct classification of 90%, a cancer specificity of 72%, and a sensitivity of 81% in the training set. Visual classification of images resulted in the correct classification of 25 of 27 patients undergoing subsequent biopsy. The two patients in error were less than 35 years of age. Overall analysis has not yet been completed.

Project Officers: Bernice T. Radovich, Ph.D., R. Quentin Blackwell, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Diagnostic Radiology Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: NCI Sera Bank Facility for the Breast Cancer Task Force

Principal Investigator: Dr. Alan Schutt
Performing Organization: Mayo Foundation
City and State: Rochester, MN

Contract Number: NO1-CB-84296
Starting Date: 9/1/77 Expiration Date: 8/31/80

Goal: To establish and maintain a storage facility for serum specimens to be used by the Breast Cancer Task Force in a program designed to search for biological markers in breast cancer.

Approach: Sera specimens will be secured from breast cancer patients, benign disease patients, controls, and a screening population under separate contracts setting up a patient resource. The material will be processed, recorded, and stored in -70°C deep freezers under easily retrievable conditions with all clinical data available. The sera will be used in the search for and verification of new breast cancer markers.

Progress: Collection and inventory methods have been developed. A special vial has been obtained and supplied to the collection areas. An operational shipping schedule has been established on a regular basis. Samples have been catalogued and systematically stored. Inventory after the first 16 months of the projects is listed:

Inventory Status--May 1, 1979	No. Patients	No. Vials
Screening Project (Columbia, MO)	5,793	59,933
Operative Patients (Grand Rapids, MI)	977	9,864
Operative Patients (Wilmington, DE)	301	2,834
TOTAL	7,071	72,631

Collected since 9-1-78 report	No. Patients	No. Vials
Screening Project (Columbia, MO)	2,968	32,821
Operative Patients (Grand Rapids, MI)	572	6,059
Operative Patients (Wilmington, DE)	225	2,030
TOTAL	3,765	40,910

Project Officers: Ihor J. Masnyk, Ph.D., Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $97,950

CONTRACT RESEARCH SUMMARY

Title: Xeromammography vs. Film Mammography--A Comparative Study

Principal Investigator: Dr. Gerald D. Dodd
Performing Organization: M.D. Anderson Hospital
City and State: Houston, TX

Contract Number: NO1-CB-43993
Starting Date: 6/28/74 Expiration Date: 3/31/79

Goal: To evaluate the relative merit of conventional film mammography vs. xeromammography in the detection of breast cancer, and to delineate differences that may suggest the use of one method over the other in certain situations.

Approach: This project is being carried out among three institutions: M.D. Anderson Hospital, Stella and Charles Guttman Breast Diagnostic Institute, and Duke University. Mammographic image formation and information transmitting characteristics of each process are being assessed by way of radiographic contrast, latitude, sensitivity, and modulation transfer function. An advisory and monitoring body set up by the Diagnostic Radiology Committee of NCI will review the technical quality of the work performed by the participating institutions.

Progress: Patient accrual ceased as of June 27, 1977. Preliminary statistical results indicate little difference in the diagnostic accuracy between the two modalities. Statistically significant differences have been observed in the objective physical parameters. Final results await completion of physicians' readings in order to perform fully balanced studies. Modulation transfer functions and Wiener spectral measurements of the DuPont Lo-Dose system and Kodak AA direct X-ray film were obtained for resolution and noise comparisons. At present, methods have not been established to determine the MTF and Wiener spectrum of the xeroradiographic system in order to make a direct comparison with the Lo-Dose system and AA film. However, a specially designed test object to compare resolution differences among Lo-Dose, AA film, and the xeroradiographic system has been used. Final analysis of the data awaits the completion of film readings and the ascertainment of histopathological data and follow-up.

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Prognostic Factors for Disseminated Cancer in Patients with Early Breast Cancer

Principal Investigator: Dr. Paul Peter Rosen
Performing Organization: Memorial Hospital
City and State: New York, NY

Contract Number: NO1-CB-74097
Starting Date: 8/1/77 Expiration Date: 7/31/80

Goal: To determine the frequency of subsequent metastatic breast cancer in a group of females with "early" invasive carcinoma; and to identify those clinical and/or pathologic features or groups of features that are indicators of a high or low risk for dissemination of carcinoma.

Approach: A study population of 250 women with primary infiltrating breast carcinomas 1.0-2.0 cm in diameter, and a subset of 100 women with malignant tumors 1 cm or less in diameter will be identified in a retrospective study (1964-1969), and followed for at least 10 years. These patients will have been treated by at least a modified radical mastectomy and have been found to have negative lymph nodes. The relationships observed between pathologic and clinical data and survival will be evaluated in order to identify those factors associated with a high or low frequency of recurrence. Patients with primary tumors 2 cm or less with positive lymph nodes will also be studied to provide a basis for comparing the distribution of various features.

Progress: All pathology records for the years 1964, 1965, 1966, 1967, 1968, and 1969 (60,617 reports) have been reviewed. Among approximately 1,800 primary mammary carcinomas treated in this hospital, we have confirmed 110 cases with a primary lesion 1 cm or less in diameter. Review of 347 cases grouped in the 1.1-2.0 cm category is now in progress. Review of clinical records has been completed for 94% of the entire series.

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $77,497

CONTRACT RESEARCH SUMMARY

Title: Longitudinal Studies of Biologic Markers in Breast Cancer Patients

Principal Investigator: Dr. Morton K. Schwartz
Performing Organization: Memorial Hospital
City and State: New York, NY

Contract Number: NO1-CB-74206
Starting Date: 8/1/77 Expiration Date: 7/31/80

Goal: To assay human breast cancer tissue and sera prior to mastectomy and throughout the clinical course of the disease for potential markers, to determine how effective such markers would be for early detection of breast cancer and its recurrence, and to gain an understanding of the relationship between the concentration of the marker substances in the individual tumor and in the serum levels of the host.

Approach: Serum and tissue specimens from patients entering for diagnosis and for primary therapy will be assayed for carcinoembryonic antigen (CEA), sialyltransferase, phosphohexose isomerase (PHI) and spermine, ferritin, lactic dehydrogenase (LDH) and its isoenzymes, glucose-6-phosphate dehydrogenase (G6PD)-tissue only as a reference, β-hCG, γ-glutamyltranspeptidase and α-fetoprotein and IgA. Total protein and DNA will also be determined to serve as points of reference. Sera specimens will be collected prior to surgery, before discharge and then sequentially, every three months the first year, and every six months thereafter. For those with no recurrences, specimens will be collected on a yearly basis. Tissue will be obtained at time of biopsy and mastectomy. Should recurrences follow, metastatic tissue would undergo the same testing procedure. During the first year, approximately 150-180 patients with primary breast cancer and an additional 50 patients with benign disease will enter the study. Twenty to twenty-five specimens of normal breast tissue (from autopsy specimens) will be assayed. Tissue and serum biochemical-immunochemical data will be compared and changes and differences related to the clinical status of the patient.

Progress: As of March 1, 1979, 249 patients had been entered into the study. Studies have indicated that there is little variability in the parameters studied in different sections of the same tissue. Cancerous tissues have concentrations in most instances much higher than normal breast tissue and for most constituents the concentrations are on the average more than 3 times the concentrations of normal tissue. The concentrations of some constituents were related to age of the patient and the hormone receptor presence in the tissue. In the first group of 138 patients 72% had at least 1 marker elevated in the blood before surgery and 51% had at least 1 marker elevated 3 months later. The patients are being followed to determine which if any combination of markers can be useful in the management of patients with breast cancer.

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $116,000

CONTRACT RESEARCH SUMMARY

Title: Evaluation of Serum LDH Isoenzymes in Women with Breast Tumors

Principal Investigator: Dr. Ervin J. Hawrylewicz
Performing Organization: Mercy Hospital
City and State: Chicago, IL

Contract Number: N01-CB-53855
Starting Date: 6/30/75 Expiration Date: 3/31/79

Goal: To determine whether serum LDH isoenzyme patterns can be utilized as a diagnostic marker for breast cancer.

Approach: Serum LDH isoenzyme patterns have been determined on 800 coded, unselected patients with breast disease, benign and malignant breast tumors (pre- and postoperative) and from 200 women clinically free of breast disease (controls). Based upon the single discriminator, LDH 5/4, isoenzyme patterns were classified as Ca or benign. LDH-derived diagnosis was subsequently correlated with the pathology report. Utilizing the decoded samples, other LDH discriminators were also evaluated.

Progress: The LDH 5/4 values (mean $\pm$ S.D.) for benign disease (i.e., fatty replacement, papilloma, fibroadenoma, fibrocystic disease, dense tissue, lipoma, and fibroadipose tissue) were significantly different from the presurgical Ca, nodes-positive group. The significance was lost when compared to the presurgical Ca nodes-negative group. Similar results were obtained with the LDH 5/1 discriminator. It was determined that increasing the blood coagulation temperature (37°C) increased LDH-5 activity twofold; LDH-1 activity remains constant. At the higher coagulation temperature, the mean LDH 5/1 value (0.34 $\pm$ 0.018) for the presurgical Ca group is significantly different from fibrocystic disease ($p=0.05$), fibroadenoma ($p=0.01$), and papilloma ($p=0.001$). Based upon the standard deviation, LDH 5/1 values less than 0.25 were classified as benign. Utilizing this value, the presurgical Ca group was 80% correctly identified. Benign diseases were correctly identified as follows: fatty replacement 100%, papilloma 100%, fibroadenoma 100%, fibrocystic disease 83%. Patients with mammography diagnosis of dysplasia and dense tissue were identified as benign in 50% and 72%, respectively. The mean LDH 5/1 ratio for the group classified as hyperplasia is similar to the presurgical Ca group. Individually, 80% of the hyperplasia patients were classified as Ca type. The high temperature (37°C) coagulated serums yielded LDH isoenzyme patterns with a high degree of correlation to the disease state. Verification of the observation is recommended.

Project Officers: Ronald B. Herberman, M.D., Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Pathology Laboratory for Cooperative Clinical Studies

Principal Investigator: Dr. Gilbert H. Friedell
Performing Organization: St. Vincent Hospital
city and State: Worcester, MA

Contract Number: NO1-CB-80704
Starting Date: 3/18/68 Expiration Date: 10/17/79

Goal: To identify and define pathologic prognostic features in cases accessioned in the Primary Therapy of Breast Cancer Study and in comparable pathologic material from British, Japanese, and Hawaiian-Japanese breast cancer patients, and to correlate this information with clinical and other laboratory data from these cases.

Approach: The pathologic material collected at the Central Pathology Laboratory from Collaborating Clinical Centers and that subsequently received from British, Japanese, and Hawaiian-Japanese sources have been and are being reviewed and correlated with clinical information and other laboratory findings. Subsequent to the initial correlative efforts, and pathologic material is and will be reviewed again in an attempt to pinpoint findings of particular importance.

Progress: The tumor type and grade, nuclear grade, and presence of blood vessel invasion at the primary tumor site, and the presence of regional lymph node involvement have been determined in 381 cases and reports of the study have been published (JNCI 53:603-607, 1975; Cancer 42:2809-2826, 1978). A report concerning sinus histiocytosis in Anglo-Japanese patients was published (Lancet 1:1228-1229, 1974) and a more detailed study of the pathology in these cases has been submitted for publication. A joint review with other pathologists of sinus histiocytosis in axillary lymph nodes from Japanese, Hawaiian-Japanese, and British breast cancer patients has been completed and a manuscript is in preparation. A detailed review of all axillary lymph nodes in radical mastectomy specimens from a series of Japanese patients treated five or more years at the National Cancer Center Hospital in Tokyo has been completed; correlation with patient survival is now in progress.

Project Officer: Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Prognostic Significance in Breast Cancer of Regional Lymph Node Immune Response

Principal Investigator: Dr. Susanna Cunningham-Rundles
Performing Organization: Sloan-Kettering Institute
City and State: New York, NY

Contract Number: NO1-CB-84228
Starting Date: 9/1/78 Expiration Date: 8/31/80

Goal: Evaluation of the relevance of regional lymph node immune response to prognosis in breast cancer. This will require an assessment of cell-mediated immune reactivity in well-standardized in vitro assays to defined antigens associated with the development of disease.

Approach: Immunological procedures will be performed on lymph nodes of 100 patients with primary operable breast cancer, using a total of 4-6 lymph nodes per patient including both negative and positive nodes, ideally from each level (I-III). In addition, control lymph nodes will be obtained from 50 other patients. The assays to be done on both peripheral blood and lymph node mononuclear cells include leukocyte migration inhibition factor (LMIF) and lymphocyte transformation tests, the latter comprising PHA, Candida, E. coli, T-antigen, and MuMTV. A third class of newer tests includes natural killer cells, suppressor cells, cell surface markers, and monocyte function assays. Clinical and demographic data as well as histopathologic data on tumor size and histologic type will be obtained. Three-year follow-up information on recurrence and survival status will be recorded. Correlation of all factors will be statistically performed.

Progress: The principal findings of this study to date may be briefly summarized as follows: Spontaneous lymphocyte-mediated cytotoxicity (SLMC) of PBL was found to be normal for most patients, and significantly increased SLMC in regional lymph nodes was observed in some patients. Corresponding increase of T_γ cells in the regional lymph node cell population was also observed. These findings are in contrast to studies of control lymph node cells where SLMC and T_γ cells were virtually absent. Increased peripheral T_γ cells were found in 50% of patients studied. Comparison of peripheral blood mononuclear cells and regional lymph node cell responses to mitogens and antigens including T antigen and MuMTV has indicated that significant differences between these responses are characteristic.

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $107,150

CONTRACT RESEARCH SUMMARY

Title: Biochemical Analysis of Human Breast Cyst Fluid (BCF)

Principal Investigator: Dr. Morton K. Schwartz
Performing Organization: Sloan-Kettering Institute
City and State: New York, NY

Contract Number: NO1-CB-53853
Starting Date: 6/30/75 Expiration Date: 9/14/80

Goal: To establish the biochemical and immunochemical composition of human BCF; to search for a marker which might be useful in understanding the formation of cysts, their treatment, and the risk of patients with cysts developing cancer.

Approach: BCF from patients with cystic mastopathy will be analyzed for polypeptide and steroid hormones, tumor-associated antigens, enzymes, lipids, minerals, and trace elements. These data will be correlated with each other and with the demographic information. Fluid will be analyzed from patients with recurrent cysts, and clinical follow-up will be obtained on all patients.

Progress: Patient entry has been completed. There have been 1,044 patients (1,830 specimens) entered into the study. Forty-two of these patients have breast cancer. About 25% of the patients have had recurrent cysts and additional specimens submitted for analysis since their initial aspiration. As previously noted, the concentrations in many BCF specimens of CEA, α-fetoprotein, β-subunit hCG, calcium, copper, zinc, total protein, albumin, cholesterol, phosphohexose isomerase, γ-glutamyltranspeptidase, β-glucuronidase, lactic dehydrogenase, amylase, dehydroisoandrosterone, androsterone, and their sulfates are many-fold the concentrations of these constituents found in serum. Correlations of BCF concentrations for many of these constituents in the same patients were found to be significant. The specimen variation was much less in specimens from the same patients obtained either at the same time or on subsequent aspiration than that from patient to patient. Notwithstanding the large BCF to serum gradient, isotope studies have indicated a slow influx and efflux of the steroids into the cyst fluid. The fluid appears to contain material both of a secretory nature and that resulting from cell breakdown. BCF contains low but detectable levels of terminal deoxynucleotidyl transferase (TdT) and DNA polymerases were observed in BCF and in supernatant from cells from BCF maintained in culture media. Biostatistical analysis has indicated that in the 40-65 years age group where the cyst occurrence is most prominent, the incidence of breast cancer in cyst patients is 625 per 100,000 as compared to 144 per 100,000 from estimates in normal women. Follow-up of patients is now proceeding.

Project Officers: Bernice T. Radovich, Ph.D., R. Quentin Blackwell, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $8,932

CONTRACT RESEARCH SUMMARY

Title: Longitudinal Studies of Biologic Markers in Breast Cancer Patients

Principal Investigator: Dr. Howard H. Sussman
Performing Organization: Stanford University
City and State: Stanford, CA

Contract Number: NO1-CB-74086
Starting Date: 8/1/77 Expiration Date: 7/31/80

Goal: To assay human breast cancer tissue and sera, prior to mastectomy and throughout the clinical course of the disease, for potential markers to determine how effective such markers would be for early detection of breast cancer and its recurrence, and to gain an understanding of the relationship between the concentration of the marker substances in the individual tumor and in the serum levels of the host.

Approach: Tumor and sera of patients presenting initially for diagnosis and primary therapy (Category 1) will be assayed for the fetoplacental proteins; i.e., placental alkaline phosphatase (PAP), human chorionic gonadotropin (hCG) α and β, human placental lactogen (hPL), and carcinoembryonic antigen (CEA), and tests on sera will be repeated sequentially every three months. Patients developing recurrences (Categories 2 and 3) will be included with a study population from the Northern California Oncology Group. Another objective of the study will be to identify new ectopically synthesized placental membrane proteins, which may be common to both trophoblasts and neoplastic breast tumor cells.

Progress: Sixty-three patients from Category 1 have been signed into the study and are being followed longitudinally throughout the course of the disease for the expression of tumor markers.

Seventy-eight patients from Category 3 have been tested for PAP and α-hCG. PAP was elevated (> 2 ng/ml) in 20% of the metastatic patients, and α-hCG was elevated (> 3 ng/ml) in 14%. Fifty-four Category 3 patients were tested for β-hCG, and 35% were elevated (> 2.5 ng/ml) for this marker. Positive patients from Category 3 are being followed longitudinally.

Assay of 16 primary tumors for PAP showed no marked production of PAP. Assay of 11 metastatic tumors from breast adenocarcinoma showed one strong PAP-producing tumor metastatic to bone. The assay for α-hCG and β-hCG in primary tumors gave a higher incidence of elevated values for α-hCG (18% > 0.5 ng/mg protein). β-hCG was minimally elevated in one tumor (2%). In metastatic tumors, 28% of the samples showed elevated α-hCG levels (> 0.5 ng/mg protein).

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $126,900

CONTRACT RESEARCH SUMMARY

Title: Biologic Characterization of "Premalignant" Human Mammary Epithelial Hyperplasias

Principal Investigator: Dr. Hanne Jensen
Performing Organization: University of California
City and State: Davis, CA

Contract Number: N01-CB-84316
Starting Date: 9/18/78 Expiration Date: 9/17/80

Goal: To establish whether angiogenesis is a reliable criterion for precancer of the human breast and to characterize by biochemical and immunological markers the type(s) of lesions that possess angiogenic capacity from those that do not.

Approach: Fresh living parenchymal structures and hyperplastic lesions are being dissected from cancer-associated breasts and from breast biopsies. All lesions are studied for their angiogenic potential by transplanting onto the iris of female rabbits. In addition, 2 thick frozen sections of lesions will be stained with fluorescent-labeled anti-human immunoglobulins G, A and M, respectively, to characterize them immunologically and with fluorescent-labeled estrogen and progesterone to detect the presence or absence of receptors to these hormones.

Progress: A total of 238 transplants have been studied to date. They were obtained from 120 lobules and lesions and dissected out of 10 breast biopsies and 3 mastectomy specimens. Angiogenesis was elicited from two lactating lobules and a lactating fibroadenoma from a recently pregnant woman and a lactating adenoma from a nonpregnant woman. Angiogenesis was consistent when 2 to 6 portions of these individual lesions were transplanted. Five of 11 hyperplastic, atypical lobules derived from a cancer-associated breast evoked angiogenesis. One of them was transplanted as 4 fragments; all elicited new vessel formation. The other 4 were transplanted as one fragment due to their smaller size. Sclerotic lobules are negative for angiogenesis to date. The immunoglobulin content of the lactating lobules from the nonpregnant woman was IgG and IgA. That of the lactating lobules from the recently pregnant woman was IgG only. One hyperplastic atypical lobule from a noncancerous breast biopsy of a 71-year-old female that failed as a transplant due to poor transplantation technique contained no immunoglobulins. The lactating lobules and the lactating adenoma did not absorb fluorescein-labeled estrogen.

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $85,550

CONTRACT RESEARCH SUMMARY

Title: Detection of Immune Complexes in Sera of Patients with Breast Cancer

Principal Investigator: Dr. M. Edward Medof
Performing Organization: University of Chicago
City and State: Chicago, IL

Contract Number: NO1-CB-84224
Starting Date: 9/1/78 Expiration Date: 8/31/80

Goal: To assay sera from patients with breast cancer or benign breast disease and normal volunteers for immune complexes (IC) employing multiple techniques for IC detection and quantitation, then to correlate presence or absence, or levels where applicable, of individual IC subpopulations with tumor progression, response to therapeutic intervention, and clinical course.

Approach: Four detection techniques for ICs (Raji-cell receptor assay, conglutinin-binding assay, solid phase Clq binding assay, and PEG-Clq precipitation assay) are established in this laboratory and will be run on sera of 40 new breast cancers, 200 benign disease patients, 84 patients with disseminated disease, and 500 normals in the first year. Serum samples will be assayed for soluble and insoluble, complement bearing, and IgG-Fc accessible ICs. Data will be analyzed according to clinical stage of patients, histologic type of tumor, histology of regional lymph nodes, presence or absence of CEA and K-casein, opsonin, and pancreatic oncofetal antigen (POA), β -human chorionic gonadotropin (β-hCG), and prolactin.

Progress: Systems have been developed and are now operational for collection, coding, and storage of blood samples; assembling pertinent clinical data; and for data analysis. IC assays have been perfected and standardized including modification of the Raji cell assay for use with conventional rabbit anti-IgG antibody to detect cell bound ICs. Preliminary measurements have been made on sera from 81 patients.

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $52,450

CONTRACT RESEARCH SUMMARY

Title: Mass Screening for Breast Cancer by Electronic Infrared Pattern Recognition

Principal Investigator: Dr. JoAnn D. Haberman
Performing Organization: University of Oklahoma
City and State: Oklahoma City, OK

Contract Number: NO1-CB-43869
Starting Date: 4/1/74 Expiration Date: 6/29/79

Goal: To design and clinically test a prototype system for mass screening for breast cancer using quantitative thermography and computer processing with automated methods of evaluation.

Approach: This project is Phase II of a proposed three-phase development program. The first phase included the following: (1) Construction of an improved thermographic instrument designed with the unique serial scan multiple element detector developed by Honeywell. This instrument provides radiometrically calibrated infrared imagery which is subsequently digitized. The instrument is equipped for direct recording on a standard video recorder and for conventional television display. (2) Initiation of a program of clinical evaluation to assess the utility of various quantitative parameters in selecting the thermographic changes of breast cancer. (3) A time-series evaluation of a group of subjects to obtain estimates of the influence of several physiological factors on the thermal patterns of the breast. Phase II consists of further analysis employing methods of computer analysis (allocation rules) capable of selecting women with cancer from those with normal breasts.

Progress: Initial months of Phase II were devoted to system improvement with incorporation of a 6 bit A/D conversion video digitizer providing "on-line" digitization of the thermal image. Presently, all thermograms are digitized "on-line." To date, 10,084 examinations including 105 cancers are included in the data base. Thermal variables (absolute temperature measurement parameters) based on earlier observations of the composition of qualitative images, breast anatomy, breast physiology, and breast heat transfer analysis have been identified. These variables (identifying features) are being examined as diagnostic indicators. A number of allocation rules (discriminating equations utilized in computor processing and automated methods of evaluation) based on a set of 400 normals, 250 benign (biopsied) cases, and 105 cancers have been developed. The task of quality review of collected data, tape reduction, and processing is nearing completion. Collection of additional cancers is progressing parallel with analysis of data to establish which allocation rules will most effectively discriminate cancer subjects from the normal population.

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Risk Associated with In Situ Carcinoma and "Precancerous" Mammary Hyperplasias

Principal Investigator: Dr. David L. Page
Performing Organization: Vanderbilt University
City and State: Nashville, TN

Contract Number: NO1-CB-74098
Starting Date: 7/15/77 Expiration Date: 7/14/80

Goal: To compare subgroups of females regarding their incidence of breast carcinoma during a 10- to 25-year interval after biopsy diagnosis of in situ carcinoma and mammary epithelial hyperplasias.

Approach: This will be a retrospective study in which slides will be reviewed and classified on over 9,000 breast biopsies performed during 1952-1968. This study population will then be contacted and follow-up information on each subject obtained regarding subsequent development of mammary carcinoma, as well as other pertinent clinical and epidemiological data. The control group will be composed of patients with biopsy but no evidence of proliferative lesions. An effort will be made to identify those specific breast lesions or combinations of lesions which are associated with an increased risk of development of breast cancer. Non-anatomic risk factors will also be correlated.

Progress: There have been 1,780 contact letters mailed with 1,206 completed questionnaires on file. Operating surgeons and their office personnel have given invaluable aid to these efforts. Surgical Pathology logbooks from three hospitals have been completely reviewed (1952-1968), creating a list of all breast operations. A data quality assurance program has been designed and implemented. Histologic classification is complete for over 6,000 biopsies. We have identified 110 biopsies with in situ carcinoma, 423 biopsies with severe hyperplasia of ductal type, 704 biopsies with moderate degree of ductal hyperplasia, and 109 biopsies with atypical hyperplasia of lobular type.

Project Officer: Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $109,200

CONTRACT RESEARCH SUMMARY

Title: Biologic Markers in Breast Cancer: Patient Resource

Principal Investigator:	Dr. Leslie W. Whitney
Performing Organization:	Wilmington Medical Center
City and State:	Wilmington, DE
Contract Number:	NO1-CB-74137
Starting Date:	9/1/77
Expiration Date:	8/31/79

Goal: To develop a Breast Cancer Task Force specimen resource for blood from breast cancer patients and controls to be used in a search for and verification of new breast cancer markers.

Approach: Thirty milliliters of blood will be collected from breast disease patients who are scheduled to undergo biopsy and/or primary surgery for breast lesions prior to surgery. Another specimen will be collected, when feasible, 5-10 days post-mastectomy from the same patient. Serum specimens will be stored at -70°C, then shipped to an NCI-designated blood bank facility with appropriate clinical data.

Progress: In the report of our first contract year (9/1/77 - 8/31/78) 92 patients were erroneously reported as entered in study. Our record shows that 65 patients were entered, 27 of those were malignant. On 7/10/78, a registered nurse was employed to coordinate the program and begin more active recruitment of participating physicians. As a result, the number of participating surgeons has increased from 9 in the first year to a total of 16 to date and recruitment efforts continue. A system for blood collection from both in- and outpatients has been established at both the Wilmington Medical Center (all divisions) and the St. Francis Hospital, so patient blood collection becomes routine among patients of participating physicians without the project nurse coordinator being present. During this reporting period (9/1/78 - 2/28/79), 112 patients have been entered into the study; 75 benign cases and 37 with malignant disease, the latter requiring two separate specimens; and 1,285 vials have been processed and shipped to the Mayo Blood Bank together with the appropriate clinical data. First year follow-up of patients with malignant disease has begun. To date, this includes three patients from whom third specimens have been collected, processed, and sent to Mayo Clinic.

Project Officers: Ihor J. Masnyk, Ph.D., Bernice T. Radovich, Ph.D.
Program: Breast Cancer Diagnosis
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Estrogen Replacement after Premenopausal Oophorectomy and Breast Cancer Risk

Principal Investigators: Dr. Dennis Slone and Dr. Samuel Shapiro
Performing Organization: Boston University
City and State: Cambridge, MA

Contract Number: NO1-CB-74099
Starting Date: 9/1/77 Expiration Date: 8/31/79

Goal: To examine the hypothesis that exposure to female hormones, particularly estrogen replacement therapy, is related to an increased risk of breast cancer, either during use or after a latent interval.

Approach: An epidemiological study of the case-control type is being implemented as part of an ongoing, hospital-based, multicenter data collection system. Cases of breast cancer and two control groups are interviewed by trained nurse-monitors in hospitals throughout the country. One control group consists of women with cancers not suspected to be related to female endocrine physiology. The second group of controls comprises hospitalized women with a variety of benign conditions. Information on lifetime drug use and relevant medical data are recorded on standard forms and transferred to computer files for analysis.

Progress: As of the end of August 1978, 377 women with breast cancer had been studied. Of these, 140 (42%) were newly diagnosed, of whom 9% were menopausal because of bilateral oophorectomy. Women with breast cancer are currently being interviewed at a rate of 600 per year.

In our data (Rosenberg et al., Am. J. Epidemiol., in press), rates of conjugated estrogen use among potential controls are 3% among premenopausal women, 15% among naturally postmenopausal women, 20% among women menopausal because of hysterectomy who retained at least one ovary, and 39% among women menopausal because of bilateral oophorectomy.

Project Officers: Elizabeth P. Anderson, Ph.D., Robert Hoover, M.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Committee

FY 79 Funds: $41,500

CONTRACT RESEARCH SUMMARY

Title: Correlations Between Dietary Factors and Pre- and Postmenopausal Breast Cancer

Principal Investigator:	Dr. Baruch Modan
Performing Organization:	Chaim Sheba Medical Center
City and State:	Tel Hashomer, Israel

Contract Number: NO1-CB-53883
Starting Date: 6/30/75 Expiration Date: 6/29/79

Goal: To carry out epidemiological investigations of selected population groups to elucidate the possible role of diet and/or nutritional status on the incidence, pathogenesis, and natural history of pre- and postmenopausal breast cancer.

Approach: A case-control study using a validated questionnaire is being conducted to investigate the following possible risk factors of pre- and postmenopausal breast cancer: dietary components; weight and height; radiotherapy treatment; breast feeding; long-time drug use; personal and family history of breast disease. The study population is composed of 720 newly diagnosed breast cancer cases (pre- and postmenopausal) of which 450 will be European and American born, 200 will be Asian-African born, and 70 will be Israeli born. There are two control groups: one drawn from hospitals, one from neighborhoods. These are matched to the cases for age, country of origin, and period of immigration to Israel. All analyses will be performed separately for ethnic groups, histopathologic category, and by menopausal status.

Progress: As of October 1, 1978, a total of 2,077 interviews have been completed: 727 cases, 688 surgical controls, 662 neighborhood controls. A total of 577 histopathological slides of cases have been reviewed by the study pathologists. Verification of previous hospitalization history of all cases and controls is being performed simultaneously. All efforts are now concentrated towards the completion of selected age-ethnic cells where the number of cases is low. Preliminary analysis on fiber consumption yielded equivocal results. This aspect and the complexity of the dietary problem were presented in a special symposium at the Twelfth International Congress on Nutrition in September 1978 in Rio de Janeiro.

Project Officer: Elizabeth P. Anderson, Ph.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Committee

FY 79 Funds: $67,000

CONTRACT RESEARCH SUMMARY

Title: Epidemiology of Benign Breast Disease

Principal Investigator: Dr. Baruch Modan
Performing Organization: Chaim Sheba Medical Center
City and State: Tel Hashomer, Israel

Contract Number: NO1-CB-74102
Starting Date: 8/1/77 Expiration Date: 7/31/79

Goal: To determine the characteristics of patients with different types of noninvasive breast lesions, with particular reference to diet and to those factors reputed to be preferentially associated with breast cancer, and to compare the findings with our similarly conducted breast cancer study.

Approach: The study consists of two phases: (a) case-control interview study of 600 cases of noninvasive breast diseases in terms of such risk factors as: dietary habits, age at first birth, menstrual history, menopausal status, parity, height and weight, familial breast cancer, selected drug history, and previous radiation exposure; and (b) an incidence study of noninvasive breast lesions in a total community. The characterization of the breast lesions will take account of the histological pattern, degree of atypia, and lymphorecticulo-endothelial response (LRE). Particular attention will be paid to the relative frequency of distinct histopathological forms in high- and low-risk subpopulations (i.e., Europeans, Asian-African born).

Progress: As of October 1, 1978, interviews have been conducted on 265 cases, 176 surgical controls, and 132 neighborhood controls. All of the interviews have been performed at home. All histopathological slides have been reviewed by at least one of the study pathologists. The frequency distributions of cases by conventional pathological criteria are as follows: fibroadenoma 53, proliferative 51, microcystic 37, combined 67, other 57; according to Black's grading: grade 1 -- 130, grade 2 -- 129, grade 3 -- 6. The pathology review shows that grade 3 is rarer than originally expected. The reason for this discrepancy is that the estimate of the frequency distribution of the various grades in the population was provisional, since no large-scale study using Black's grading had previously been undertaken. In conjunction with the incidence study, which is planned for next year, we will have a larger pool of cases available so that a sufficient number of grade 3 cases can be obtained.

Project Officers: Elizabeth P. Anderson, Ph.D., William Blot, Ph.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Committee

FY 79 Funds: $62,980

CONTRACT RESEARCH SUMMARY

Title: Genetic Polymorphisms in High- and Low-Risk Breast Cancer Families

Principal Investigator: Dr. Henry T. Lynch
Performing Organization: Creighton University
City and State: Omaha, NE

Contract Number: NO1-CB-44003
Starting Date: 6/17/74 Expiration Date: 6/16/79

Goal: To investigate histocompatibility antigens and other genetic polymorphisms in high- and low-risk breast cancer families. To determine whether high incidence of breast cancer in certain extended families can be attributed in part to genetically determined susceptibility or only to shared environmental influences; that is, to determine if there is a gene or genes influencing breast cancer susceptibility and, if so, to elucidate its mode of transmission.

Approach: The mathematical techniques of segregation and linkage analysis are being applied to 21 large kindreds with documented high incidence of breast and associated cancers. The observed pattern of cancer distribution in each family is compared to the pattern expected were the disease determined by (1) an autosomal dominant allele, (2) an autosomal recessive allele, (3) a sex-linked allele, or (4) purely environmental factors. For those families for which one of the genetic models best fits the observed distribution of cancer, linkage analysis is applied to confirm the existence of the hypothetical susceptibility allele by mapping its chromosomal location with respect to each of a large number of clinically innocuous marker genes.

Progress: A total of 21 families have been studied by segregation analysis; for 15 of these, genotypes of informative relatives have been determined for each of 18 marker loci. Four of the families at high risk for breast cancer each also include one or more cases of ovarian cancer. For these "breast-ovary" families, a genetic model for transmission of breast and ovarian cancer susceptibility best fits the data. For five families with breast and endometrial or cervical cancer, an autosomal dominant model for susceptibility to any female genital cancer provides the best fit. For three families with breast and stomach or colorectal cancers, an autosomal dominant model for susceptibility to breast cancer alone best fits. For two families with breast, brain, and adrenocortical cancers, sarcomas, and leukemia, a different autosomal dominant model (with very early age of disease onset) best explains the data. For one family, an environmental model best explains the distribution of cancer. For each of the four groups of families consistent with a genetic hypothesis, we are currently testing the putative susceptibility gene for linkage with each marker locus.

Project Officers: Elizabeth P. Anderson, Ph.D., Joseph Fraumeni, M.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Committee

FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Epidemiologic Characteristics of Pre- and Postmenopausal Breast Cancer

Principal Investigator: Dr. David C. Deubner
Performing Organization: Duke University
City and State: Durham, NC

Contract Number: N01-CB-63996
Starting Date: 6/30/76 Expiration Date: 6/29/79

Goal: The goal is to define the epidemiologic characteristics of breast cancer in pre- and postmenopausal women and to relate these characteristics to the pathobiology of the tumor as manifested by estradiol and progesterone receptors, histology, and cellular ultrastructure.

Approach: This case-referent study is of breast cancer patients undergoing surgery at university and community hospitals, surgical and medical patients not having breast or gynecological surgery at the same hospitals, and subjects from the community. Data collection is by interview of all subjects and review of charts of hospital patients. Laboratory studies on tissue and serum from breast cancer patients include quantitative estrogen and progesterone receptor determinations, evaluation of histology and ultrastructure by a standardized protocol, and estrogen and progesterone serum determinations. Statistical analyses will estimate the relative strength of association between breast cancer in pre- and postmenopausal women and such factors as age of menarche, age of first delivery, obesity, and family history of breast cancer. Associations between risk factors and tumor cell biology and morphology will be sought by comparing the laboratory results in women with and without single or multiple risk factors.

Progress: As of January 24, 1979, 539 women with breast cancer had been entered in the study and satisfactory interviews and pathologic materials obtained from at least 88%. Two hospital referent subjects, matched by race and age, have been interviewed for each case entered. The community referent group is a random sample of women at least 30 years of age who live in the piedmont and plains regions of North Carolina. Approximately 1,500 of the anticipated 1,580 interviews have been completed since the field work began in March 1977. Laboratory procedures are being completed according to protocol as material is obtained from the participating pathologists. Data entry into the computer is virtually complete and data analyses are under way.

Project Officers: Elizabeth P. Anderson, Ph.D., Gordon B. Cutler, M.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Committee

FY 79 Funds: $169,700

CONTRACT RESEARCH SUMMARY

Title: Endocrine Events at the Time of First Pregnancy

Principal Investigator: Dr. John R. K. Preedy
Performing Organization: Emory University
City and State: Atlanta, GA

Contract Number: NO1-CB-74101
Starting Date: 8/1/77 Expiration Date: 7/31/79

Goal: It is proposed that the protective effect of an early first pregnancy against breast cancer is due to a change in the hormonal environment following that event. We propose to investigate and characterize such a change.

Approach: Nulliparous subjects in the 18-22 age range who have not taken birth control pills, who have a history of normal regular menses, and who are planning pregnancy in the next 1-2 years will be selected (Group A). Similar subjects will be chosen who do not plan to become pregnant in the next 1-2 years (Group B). Similar subjects to the above but in the age range 30-40 would also be selected (Groups C and D). All groups would have the following carried out in the early follicular phase of the menstrual cycle: plasma gonadotropins, estrogens, progesterone, androgens; urine estrogens and androgens; plasma protein-steroid binding studies; perphenazine-prolactin stimulation test; LRH-LH stimulation test. Results from Groups A, B, C and D would be compared by appropriate analysis of variance techniques to determine significant contrasts among the four groups.

Progress: The extreme and fully recognized difficulty in identifying and recruiting eligible volunteers in Groups A and C continues. In consequence the most strenuous efforts have been made to increase the number of volunteers in these categories. Saturating media campaigns on radio, television, newspapers, etc. have been carried out for the Atlanta area and state-wide. As a result, as of 5/31/71, we now have the following total numbers of subjects in the program: Group A 45 (initial study done on 40 and 4 now pregnant), Group B 14 (initial study done), Group C 25 (initial study done on 22, 1 now pregnant), Group D 14 (initial study done). Subjects in Groups B and D are, of course, easier to obtain, and a pool of these potential controls is already available. Some attempt will be made to match controls roughly with women in Groups A and C who succeed in becoming pregnant.

Project Officers: Elizabeth P. Anderson, Ph.D., Richard Knazek, M.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Committee

FY 79 Funds: $121,500

CONTRACT RESEARCH SUMMARY

Title: Pathogenetic Models of Malignant and Premalignant Disease of the Breast

Principal Investigators: Dr. John A. H. Lee and Dr. Suresh Moolgavkar
Performing Organization: Fred Hutchinson Cancer Research Center
City and State: Seattle, WA

Contract Number: NO1-CB-74100
Starting Date: 9/1/77 Expiration Date: 8/1/79

Goal: To translate hypotheses, such as the multistage theory, about the development of breast cancer into mathematical terms, and to test the predictions of these against a range of age-specific incidence rates of the disease.

Approach: The behavior of the incidence curves generated by models will be studied under perturbations of the parameters. Maximum likelihood estimations will be used to fit the model to population data which have been statistically analyzed to allow for the effects of secular trends on age distribution.

Progress: Two papers stemming from the contract have appeared in JNCI. The first shows that the age-specific incidence and mortality curves of male breast cancer in Japan and the U.S. behave like those of other common epithelial tumors, and do not exhibit the peculiarities of the incidence curves of female breast cancer. The second paper discusses the Armitage-Doll approximation to the multistage theory of carcinogenesis, shows that under certain conditions this approximation is inappropriate, and suggests a better one. A third paper, describing new methodology for adjustment of temporal trends in cancer and applying this to female breast cancer data, is to appear in JNCI; after such adjustment, the incidence curves for female breast cancer from various geographic locations have remarkably similar shapes. Even in two Japanese prefectures from which data were available the curves continue to rise after menopause.

Solution of the equations of the theoretical model has been undertaken. This model differs from the usual models in that mutations are associated with cell divisions and the target cell population is allowed to increase in size (as it certainly does in childhood and adolescence). Preliminary results show that a two-mutation model is capable of generating incidence curves of diverse kinds. For adult tumors, in particular for female breast cancer, a third stage, that of promotion, is required, where hormones are postulated to act. The fitting of these curves to population data has yet to be undertaken.

Project Officers: Elizabeth P. Anderson, Ph.D., Sylvan Green, M.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Committee

FY 79 Funds: $83,690

CONTRACT RESEARCH SUMMARY

Title: Lipid and Cholesterol Levels in Relation to Human Breast Cancer Risk

Principal Investigator: Dr. Gary D. Friedman
Performing Organization: Kaiser Foundation Research Institute
City and State: Oakland, CA

Contract Number: NO1-CB-84238
Starting Date: 8/7/78 Expiration Date: 8/6/79

Goal: To explore a possible etiologic role of dietary fat in breast cancer, by examination of breast cancer incidence in relation to previous levels of cholesterol and/or lipids.

Approach: We plan to compare incidence rates of breast cancer and associated cancers in groups of women defined by their serum cholesterol and/or lipid levels at a time prior to their diagnosis. Using the resource of data from the Northern California Kaiser Health Plan's multiphasic health check-ups (95,000 women between 1964 and 1976), we estimate that more than 250 cases of breast cancer will be available for review. Medical data on cholesterol and, for some, on lipid levels will be available. Other known and available risk factors and possible confounding variables, such as obesity and ethnic background, will be considered in analysis.

Progress: In the first six months of the project, data on women in the population at risk have been isolated from the several computer-stored files of persons who have taken the multiphasic health check-up (MHC). We have identified 96,475 women in the age group over 15 years of age who have taken a MHC and who we are calling the population at risk. All cases of cancer of the breast as well as of the ovary, uterine corpus, rectum, and colon have been identified from the file of Kaiser Foundation Health Plan (KFHP) hospital discharges and annual disease indices. Medical records of approximately 4,000 possible cases were manually reviewed. At this time we have identified 1,330 cases of breast cancer by manual count which occurred among these women in the post 1964 period. Before data analysis can begin we have yet to add any additional cancer cases recorded by the California Tumor Registry and not in the KFHP file and to examine possible differences in the cholesterol level determination that may have arisen from a methodologic change in 1968.

Project Officers: Elizabeth P. Anderson, Ph.D., Helen Hubert, Ph.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Committee

FY 79 Funds: $67,000

CONTRACT RESEARCH SUMMARY

Title: The Relationship Between Thyroid Disease and Breast Cancer

Principal Investigator: Dr. Farahe Maloof
Performing Organization: Massachusetts General Hospital
City and State: Boston, MA

Contract Number: N01-CB-84230
Starting Date: 9/30/78 Expiration Date: 9/29/79

Goal: To examine the possible association of breast cancer with prior thyroid dysfunction.

Approach: This will be a retrospective cohort study designed to measure the rates of breast cancer in women with and without prior thyroid disease. A mortality study will follow 7,000 women with thyroid disease, seen at the Thyroid Clinic of the Hospital between 1921 and the present, and 7,000 matched population controls; 2,000 women seen at the Clinic but without identified thyroid disease will also be followed. Rates will be compared in women with various types of thyroid disease. A morbidity study will follow 1,600 women with previous thyrotoxicosis; this will include 500 women who became hypothyroid following the treatment and 500 who did not. Rates of breast disease will be compared according to mode of treatment as well as externally compared with rates from the Connecticut Tumor Registry.

Progress: Mortality Study: The card file of patients treated at the Thyroid Clinic was reviewed. From the 45,000 names, 13,410 women satisfied the study criteria, almost twice as many women as had been anticipated, allowing us to increase the sample size. This included 3,142 women with no thyroid disease. The information was coded, keypunched, and entered into the computer. To date, 2,000 names have been chosen from the Massachusetts Residents' Lists as potential controls matched for age and neighborhood.

Morbidity Study: A roster of 1,955 women from the USPHS Thyrotoxicosis Follow-up Study has been compiled and is being entered into the computer. A preliminary death search found 375 women dead. A letter and questionnaire are being mailed to each woman still indicated as alive. During the next six months, the questionnaire will be analyzed and patients will be selected for physical examinations.

Project Officers: Elizabeth P. Anderson, Ph.D., Bruce Nisula, M.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Committee

FY 79 Funds: $141,400

CONTRACT RESEARCH SUMMARY

Title: Lipid Levels and Cholesterol Metabolism in Relation to Human Breast Cancer Risk

Principal Investigator: Dr. Angelos E. Papatestas
Performing Organization: Mount Sinai School of Medicine
City and State: New York, NY

Contract Number: NO1-CB-84318
Starting Date: 9/30/78 Expiration Date: 9/29/79

Goal: The role of lipids and cholesterol in breast cancer risk is to be explored to evaluate whether dietary or metabolic factors affect causal mechanisms for the development of breast cancer and whether these factors act on genetically susceptible substrates either systemically or locally at the target organ.

Approach: A pair-wise matched case control study. Data from the past medical and family history, dietary habits, and laboratory data on lipid levels and cholesterol metabolism in patients with breast cancer and in controls (patients and volunteers) will be analyzed. Possible correlations between these parameters and epidemiological risk factors, or clinical and histopathological parameters, will be sought. Data on enzymatic activity, on adipose tissue cellularity, and on estrogen and progesterone receptors will also be analyzed.

Progress: Accrual of patients is proceeding in a satisfactory way, and we expect to enter between 250 and 300 patients in the study during the first year. No patient approached in the first six-month period has refused to participate in the study. In the first three months, data were collected for plasma cholesterol, triglycerides, fatty acids, estrogen and progesterone receptor proteins, and samples were collected for adipose tissue cellularity and enzymatic activities. We also started collecting data for plasma cholesterol kinetics, fecal steroid degradation products, and tissue steroids and fatty acids of cancerous and normal tissue. Analysis of preliminary data was initiated in March of 1979. The investigative team is meeting once a week to review progress of the study and identify any problems related to the scientific aspects. The collaboration between the members of the team has been excellent and the rapport between the investigators and the patients has been outstanding, as reflected by the 100% participation of those patients approached for the study.

Project Officers: Elizabeth P. Anderson, Ph.D., Kenneth Lippel, Ph.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Committee

FY 79 Funds: $198,000

CONTRACT RESEARCH SUMMARY

Title: Genetic Polymorphisms in High- and Low-Risk Breast Cancer Families

Principal Investigator: Dr. Frans J. Cleton
Performing Organization: The Netherlands Cancer Institute
City and State: Amsterdam, The Netherlands

Contract Number: NO1-CB-43940
Starting Date: 6/18/74 Expiration Date: 6/30/79

Goal: To study linkage of certain genetic markers with breast cancer in an attempt to select high-risk groups in the population for the development of breast cancer.

Approach: HL-A haplotypes will be studied in families with a high incidence of breast cancer. Certain genetic markers located on the 6th chromosome and the red blood cell groups will also be investigated. The study of red cell isoenzymes, immunoglobulins, and chromosomal polymorphisms have been stopped after the analysis of data on 14 families. The search for cross-reacting antigens with mouse MTV-sera, yielding negative results, has also been stopped. Plasma-prolactin levels will be measured in breast cancer patients and their daughters, with special emphasis on diurnal fluctuation and variation during the menstrual cycle. Because multiple case families with sufficient living affected members in two generations are rare, it was decided also to investigate pairs of sisters with breast cancer. Linkage of breast cancer with HL-A can be determined by comparing similarities in HL-A haplotypes in these pairs with the expected similarity.

Progress: Initial linkage studies have been carried out on 700 members from 14 high-risk families, including 60 patients with breast cancer; these initial analyses showed no significant evidence of linkage between breast cancer and any of the 70 genetic markers that were studied. A computer program for linkage analysis, especially designed for incomplete family data, was used and the results were expressed as lod scores. Further linkage analyses, allowing for variable age of onset, in the model, are now in progress in collaboration with Dr. Robert Elston at University of North Carolina. These results will be compared to and pooled with the results of similar analyses on the data of Dr. Henry Lynch and Dr. Mary Claire King (contract #44003). Ten sister pairs have been typed for HL-A in addition to the family studies. Studies of prolactin in healthy female relatives in these families showed an irregular distribution of plasma levels, probably related to an altered diurnal regulation. These studies will be extended, including the variation of plasma prolactin during the menstrual cycle.

Project Officers: Elizabeth P. Anderson, Ph.D., Joseph Fraumeni, M.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Committee

FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Interrelationships Among Diet, Steroid Hormone Metabolism, and Human Breast Cancer

Principal Investigator: Dr. Marvin A. Kirschner
Performing Organization: Newark Beth Israel Medical Center
City and State: Newark, NJ

Contract Number: NO1-CB-84229
Starting Date: 9/30/78 Expiration Date: 9/29/79

Goal: To investigate steroid hormone production rates and metabolism in relation to obesity and dietary patterns.

Approach: This will be an in-depth study of steroid hormone production rates, metabolism, and excretion in a cohort of obese American women undergoing drastic weight reduction and diet manipulation. Forty obese, but otherwise healthy, and normally menstruating women will be studied prior to weight reduction, after weight loss and stabilization on a low carbohydrate, low fat diet, and after isocaloric exchange to a more typical high carbohydrate, high fat American diet. The studies will examine androgen, estrogen, and cortisol production rates, interconversions, and peripheral metabolism. Values will be compared under the three sets of conditions and further compared with those in lean, age-matched controls.

Progress: During the start-up phase of this study, patient recruitment and volunteer (control) recruitment was begun. Formalized diet regimens were completed, and the double-isotope techniques for measuring urinary metabolites were established. As of 3/31/79, we have performed metabolic clearance studies, blood production rates and urinary estrogen production rates on 26 obese women at the point of their entrance into our weight loss program. To date, six clearance studies have been completed and generally show elevated values. Ten women have remained actively in the program, of whom three have lost 50-100 lbs. to date. Despite the higher-than-expected dropout rate, we are analyzing the hormone production studies done on all the obese women. We have actively recruited normal lean women to serve as controls. To date, 17 women have completed the initial study; 8 have completed the high protein diet and have been re-studied, and 6 have completed all three parts of the study.

Project Officers: Elizabeth P. Anderson, Ph.D., Lynn Loriaux, M.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Committee

FY 79 Funds: $160,800

CONTRACT RESEARCH SUMMARY

Title: Relation of Intestinal Flora to Breast Cancer in Humans

Principal Investigator: Dr. Sherwood L. Gorbach
Performing Organization: New England Medical Center
City and State: Boston, MA

Contract Number: N01-CB-74104
Starting Date: 7/1/77 Expiration Date: 7/30/79

Goal: To determine whether diet and other risk factors influence the metabolism of estrogens by bacteria in the large bowel.

Approach: The estrogen profiles will be determined in the serum, urine, and feces of young and older women eating "Western" or vegetarian diets, and in women with a history of breast cancer. The ability of the fecal flora to metabolize estrogens will also be studied. An analysis of fecal metabolic activity will be done for the different experimental groups. Novel fecal metabolic transformations of estrogens and the possible role of these transformations in the etiology of breast cancer will be investigated.

Progress: In the first year 33 women have been enrolled in this study. Between six and eight women in each of the dietary and age groups are now participating in the study. In addition, four breast cancer patients are providing specimens. A medical and dietary history has been taken on each subject. Standard clinical chemistry tests have also been performed. Samples of 72-hour urine, feces, and blood have been collected twice from all 33 subjects; 75% of the volunteers have completed a third collection period. A total urine volume and creatinine concentration have been measured. The total fecal weight, percentage water content, and concentration of fecal protein have also been determined. The urinary levels of estrone, estradiol, estriol, estriol-3-glucuronide, and estriol-16-glucuronide have been measured for 28 subjects. The concentrations of fecal estrone, estradiol, and estriol have also been measured for 28 subjects. Serum estrone, estradiol, testosterone, and androstenedione-Δ^4 dehydrogenase activities have been assayed. Currently 28 additional specimens given during collection period two are being analyzed for the same parameters. The formation of enzymatic products has been measured by gas-liquid chromatography. More than 164 enzymatic assays have been completed. Computer analysis of the dietary histories revealed a significant difference in the intake of total carbohydrate, animal protein, and animal fat between the vegetarian and omnivore groups. This analysis clearly indicates we have satisfactorily selected vegetarians and omnivores for this study.

Project Officers: Elizabeth P. Anderson, Ph.D., Carl Smith, Ph.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Committee

FY 79 Funds: $102,500

CONTRACT RESEARCH SUMMARY

Title: Epidemiology of Minimal Breast Cancer in Initially Asymptomatic Women

Principal Investigator: Dr. Bernard S. Pasternack
Performing Organization: New York University
City and State: New York, NY

Contract Number: NO1-CB-74103
Starting Date: 7/15/77 Expiration Date: 7/14/79

Goal: Investigate by means of risk factor analysis whether minimal breast cancer in initially asymptomatic women is essentially different from clinical breast cancer or whether it is an early manifestation of the same disease; identify risk factors associated with tumor aggressiveness.

Approach: We will obtain and analyze demographic and medical data recorded at the Guttman Breast Diagnostic Institute which since 1968 has been offering free breast examinations to women living in New York City. The study group will consist of an estimated 880 valid cases detected through the end of 1978 and a random selection of approximately 2,700 controls. Two independent histopathologic diagnoses will be performed. Cases will be compared according to three tumor criteria: (a) level of invasiveness, (b) level of aggressiveness (prevalent vs. incident cases), and (c) histological type. Controls will be used to confirm relative risk estimates for study factors. Comparison of all cases to controls should result in estimates of relative risk within the range found at other screening centers, whereas the comparison of clinical cases to controls should provide estimates within the same order of magnitude as registry data.

Progress: Protocols have been established for all phases of the study: collection of demographic and screening data, pathology review, and mammography review. Basic identification forms have been coded and keypunched for all 1,146 cases diagnosed through October 1977. Examination code forms for about 1,140 participants have been coded and, of these, about 480 keypunched. Initial and recall histories have been coded for about 860 participants and, of these, about 360 keypunched. Pathology material has been requested for about 180 women, received for about 100, and reviewed at NYU for about 50. An equal number of mammography films have been reviewed. Computer programs for data editing have been developed.

Project Officers: Elizabeth P. Anderson, Ph.D., Benjamin Hankey, Sc.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Group

FY 79 Funds: $112,400

CONTRACT RESEARCH SUMMARY

Title: Epidemiologic Characteristics of Medullary and Lobular Breast Cancer

Principal Investigator:	Dr. Paul P. Rosen
Performing Organization:	Sloan-Kettering Institute
City and State:	New York, NY

Contract Number: NO1-CB-63997
Starting Date: 6/30/76 Expiration Date: 6/29/79

Goal: To identify and describe morphologic features of human female mammary carcinoma which are significantly related to known risk factors for the development of such carcinomas.

Approach: Detailed epidemiologic information will be obtained by interview from women with mammary carcinoma and correlated with pathologic features of the tumors. The analysis of the data will emphasize comparison of medullary and lobular carcinoma with duct carcinoma but will also permit the study of morphologic features not limited to one tumor type.

Progress: A total of 1,227 patients have been accessioned into the study. Among the cases reviewed, 81 women were found to have only medullary carcinoma, 38 only in situ lobular carcinoma, and 59 only infiltrating lobular carcinoma. In addition, 42 patients had lobular carcinoma (38 invasive, 4 noninvasive) combined with another type of carcinoma, and 11 patients had medullary carcinoma associated with another type of carcinoma. Interviews have been conducted with 98% of the patients. Data verification has been accomplished for 75% of prior medical and surgical treatments and additional information continues to be received. All currently available data on each patient have been entered into the computer. Analysis of correlations between morphologic and epidemiologic observations is in progress.

Project Officers: Elizabeth P. Anderson, Ph.D., Roger Connelly
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Committee

FY 79 Funds: $84,750

CONTRACT RESEARCH SUMMARY

Title: Correlations Between Morphology and Epidemiology of Breast Cancer

Principal Investigator: Dr. Björn Stenkvist
Performing Organization: University Hospital
City and State: Uppsala, Sweden

Contract Number: N01-CB-53968
Starting Date: 6/30/75 Expiration Date: 6/30/79

Goal: To ascertain possible correlations between morphologic variables such as cytologic and histopathologic classifications and established epidemiological risk factors of breast cancer.

Approach: Within a clearly defined area and population (4 Swedish counties) 181 breast cancer cases occurred during a defined study period (5 months). From 179 of these patients both epidemiological and morphological data could be obtained. From 179 exactly age-matched controls epidemiological data were recorded in the same way. The tumors were characterized by computerized nuclear morphometry (CNM) of the cell populations in the tumors as well as by conventional morphology, resulting in an objective atypia measure, the CNM value.

Progress: Conventional pathological classification resulted in the definition of six subgroups; 62% of the cases were in a "not otherwise specified" category, 17% low differentiation (Ackerman group IV), 7% lobular, 5% mucinous, 3% papillary, 2% medullary, and the rest inoperable cases (2%) or "rare tumors" (4%). Comparison between patients and controls showed no significant differences in age at menarche, age at first birth, age at menopause, number of children, height, weight, obesity, or frequency of diabetes or hypertension. These results differ from most earlier reports, possibly because the controls here were selected from the whole female population instead of hospitalized patients. Heredity and frequency of prior breast disease were significantly higher in the patient group. Data on thyroid hormone levels suggested that altered thyroid function in the patients was probably due to a change in the peripheral conversion of thyroxin. The patients had higher serum concentrations of esterone, androstenedione and testosterone, and a lower level of sex-hormone-binding globulin. No obvious differences in the epidemiological characteristics could be discerned among different conventional morphological subgroups. The CNM value was positively correlated with biological parameters such as mitotic rate and had significantly higher reproducibility than subjective systems for classification or grading. Patients treated with heart glycosides had a lower CNM value than the others ($P < 0.0001$), indicating an effect on the chromatin pattern in the tumor. Further study is emphasizing epidemiological analyses and factors influencing survival.

Project Officers: Elizabeth P. Anderson, Ph.D., Cecil Fox, Ph.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Committee

FY 79 Funds: $30,000

CONTRACT RESEARCH SUMMARY

Title: Epidemiologic Characteristics of Pre- and Postmenopausal Breast Cancer

Principal Investigator:	Dr. Ralph S. Paffenbarger
Performing Organization:	University of California
City and State:	Berkeley, CA

Contract Number: NO1-CB-63998
Starting Date: 6/30/76 Expiration Date: 6/29/79

Goal: This work identifies host and environmental characteristics that predispose to the development of breast cancer in women of all ages, and contrasts the findings between premenopausal and postmenopausal women.

Approach: Using epidemiologic methods of case-control design, we are conducting structured interviews with women in the San Francisco Bay area, totaling over 2,500 breast cancer patients and 5,000 control women. Characteristics to receive special attention as potential precursors of breast cancer include age, catamenial and childbearing events, indicators of early fertility, exogenous hormone and drug use, sociocultural factors, family history of cancer, radiation exposure, and various physical measurements. Extent of disease (staging), histological diagnoses, and survival determinations are being established.

Progress: Interviews have been completed on 3,985 study subjects, 1,426 of whom are breast cancer patients of all ages and 2,559 of whom are age-, race-, and hospital-matched control patients. About 24 percent of the breast cancer patients are premenopausal. All interviews have been coded and keypunched for data processing. A series of computer-based editing filters have been programmed to check the validity and consistency of the data. Procedures to recode and update edited records have been instituted; editing is nearly complete on all subjects. A general computer program has been constructed to perform summary chi square analyses with adjustment for confounding factors, starting from primary data and user-prepared control cards. Documentation of the program has been prepared. Histological diagnoses have been made on 447 breast cancer patients; these data have been coded and keypunched. Survival follow-up of breast cancer patients has been completed through December 1977. Preliminary analyses have been made on a subset of study subjects examining potential characteristics of high risk in pre-, para-, and postmenopausal women.

Project Officers: Elizabeth P. Anderson, Ph.D., Bruce Stadel, M.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Committee

FY 79 Funds: $79,700

CONTRACT RESEARCH SUMMARY

Title: Epidemiology of Benign Breast Disease

Principal Investigator: Dr. Gary H. Spivey
Performing Organization: University of California
City and State: Los Angeles, CA

Contract Number: NO1-CB-74202
Starting Date: 8/15/77 Expiration Date: 8/14/79

Goal: To examine the epidemiology of major types of benign breast disease and to compare identifiable risk factors for benign diseases with known risk factors for breast cancer. To examine a histologic classification scheme based on grading of cellular atypia in comparison to the conventional histologic classification.

Approach: Women with first biopsies for benign breast disease in participating hospitals will be admitted to the study by a sampling mechanism designed to represent the spectrum of histologic types. A subcategory of women with breast cancer will be included. Two types of controls will be used, a hospital control and a friend control. Information on epidemiologic characteristics will be obtained by questionnaire. Epidemiologic characteristics of different histologic types of benign breast disease will be compared to each other and to those of breast cancer.

Progress: The study includes seven hospitals doing breast biopsies in the Coastal Health Services Region; procedures have been established for identification of cases and of hospital controls. Pathology slides are being classified according to both traditional pathologic classification and the Black-Chabon grading of cellular atypia. To date, we have found a lower than expected number of benign biopsies, the ratio of benign to malignant biopsies being about 2:1 in our hospitals instead of the expected 6:1. We have attempted to solve this problem by adding two new hospitals (and potentially a third), expanding the eligible area for residence beyond the coastal region, and extending the starting point for eligibility back to 1977. We will keep track of both the place of diagnosis and of residence so that population based estimates may still be made for the coastal region. A questionnaire has been developed seeking responses on a wide range of topics including environmental influences; familial cancer patterns; medical, maturation, and reproductive history; and various other endocrine-related subjects. Interviewing was seriously delayed by the necessity for OMB clearance of the questionnaire; to date, we have interviewed approximately 200 women. All interviews can be keypunched as completed. We are attempting to make up some of the lost time by increasing the case selection efforts, but we anticipate the necessity of a one-year extension of the contract to complete the project.

Project Officers: Elizabeth P. Anderson, Ph.D., B. J. Stone, Ph.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Committee

FY 79 Funds: $191,200

CONTRACT RESEARCH SUMMARY

Title: Further Linkage Analysis of Families with High Incidence of Breast Cancer

Principal Investigator: Dr. Mary Claire King
Performing Organization: University of California
City and State: San Francisco, CA

Contract Number: NO1-CB-84274
Starting Date: 6/1/78 Expiration Date: 11/30/79

Goal: To confirm the existence of postulated alleles influencing cancer susceptibility in certain families with very high breast cancer incidence.

Approach: In collaboration with Dr. R. C. Elston, University of North Carolina, we are using maximum likelihood methods of linkage analysis to map hypothetical alleles for breast cancer susceptibility with respect to seven newly developed marker loci. The 15 families under investigation, and the genetic models which best explain the distribution of breast cancer in each, have been developed in the course of our collaboration with Dr. H. T. Lynch (contract CB-44003, this volume). The present project strengthens that approach by adding markers not available at the time of the original contract.

Progress: Of the seven new genetic markers to be investigated, we have completed laboratory analysis of five. We have sent these results to North Carolina for testing chromosomal linkage with the "susceptibility alleles" whose existence was suggested by segregation analyses. We are currently screening the sixth marker (protease inhibitor) and are refining screening techniques for the last (peptidase A).

Project Officer: Elizabeth P. Anderson, Ph.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Committee

FY 79 Funds: $7,009

CONTRACT RESEARCH SUMMARY

Title: Benign and Noninvasive Breast Disease in Populations at Different Risk for Breast Cancer

Principal Investigator: Dr. Nicholas Petrakis
Performing Organization: University of California
City and State: San Francisco, CA

Contract Number: NO1-CB-84319
Starting Date: 9/30/78 Expiration Date: 9/29/79

Goal: To ascertain the prevalence and spectrum of clinically palpable benign breast disease across ethnic groups whose breast cancer rates are known to vary and to explore possible associations with known or suspected breast cancer risk factors. In addition, to investigate cytologic measures which might serve to distinguish women with benign breast disease.

Approach: Data regarding breast findings at clinical examination will be analyzed in relation to sociodemographic and medical information collected in a large regional breast screening program for white, Black, Chinese, Japanese, and Latin women. For a subset of these women, breast fluid specimens obtained through nipple aspiration will be examined cytologically and findings correlated with clinical breast disease status. A separate attempt will be made to collect similar information from women of various ethnic groups in the general population, in a pilot effort to assess the feasibility of a community-based survey of benign breast disease.

Progress: During the first six months, we have focused upon the existing study population of approximately 10,000 women enrolled in the Breast Screening Center of Northern California located in Oakland. For Japanese, Black, Chinese, and a sample of white women in this population, we are collecting the following for data analysis: (1) the results of an interview or self-administered questionnaire regarding social and medical history; (2) breast fluid specimens for cytologic analysis; (3) the results of a "Clinical Breast Findings" form; and (4) all data routinely collected as part of the screening center program, including mammographic and detailed breast physical findings. All of these data will be analyzed as they relate to benign breast disease. In addition to the screening center efforts, we are analyzing existing data collected since 1972 for an ongoing series which now includes nearly 5,000 women who have participated in epidemiologic studies of breast secretion and breast cancer. Breast findings have been routinely recorded for this population, along with information on known or suspected risk factors for breast cancer. Breast secretory activity and the results of cytologic analyses, as well as other demographic and reproductive characteristics will be compared in women with breast disease and normal women.

Project Officers: Elizabeth P. Anderson, Ph.D., John Mulvihill, M.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Committee

FY 79 Funds: $30,000

CONTRACT RESEARCH SUMMARY

Title: Role of Dietary Factors and Noncontraceptive Estrogens in Breast Cancer

Principal Investigator: Dr. Abraham M. Y. Nomura
Performing Organization: University of Hawaii
City and State: Honolulu, HI

Contract Number: N01-CB-53884
Starting Date: 6/30/75 Expiration Date: 6/29/79

Goal: To determine in postmenopausal women if there is an association of breast cancer with specific dietary factors and the use of menopausal estrogens.

Approach: A case-control approach is being utilized in which a questionnaire is employed to collect information on past history of drug usage (including menopausal estrogens) and usual weekly dietary intake. Breast cancer cases from three selected populations (ages 45-74) are identified: (1) the Caucasians in Hawaii who are at high risk for breast cancer; (2) the Japanese in Hawaii who are at intermediate risk; and (3) the Japanese in Fukuoka, Japan, representing a low-risk population. For each case, a neighborhood and hospital control matched by race and age is interviewed. Attempts are made to verify the drug history with the subject's personal physicians and a subsample of the study population receives a repeated dietary interview to assess its reliability.

Progress: The study originally aimed to interview 200 cases and 400 controls in each of the three ethnic groups. The Japanese portion of the study has been completed (as of 3/31/79), but required the addition of two more hospitals to obtain the requisite number of cases; we expect to interview in Japan a final total of 215 cases, plus their respective controls, and have interviewed (as of 10/31/78) 210 cases, 198 hospital controls, and 208 neighborhood controls. The Japanese and Caucasian populations in Hawaii are not yielding the anticipated numbers of cases, and there are no additional hospitals to include. As of 11/30/78, a total of 228 cases have been interviewed in Hawaii, plus 211 hospital controls and 200 neighborhood controls. It is hoped that an additional 18 months of the study will increase the totals to 195 Japanese and 160 Caucasian cases in Hawaii.

Project Officers: Elizabeth P. Anderson, Ph.D., David Levin, M.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Committee

FY 79 Funds: $84,900

CONTRACT RESEARCH SUMMARY

Title: Lipid and Cholesterol Levels and Breast Cancer Risk in Tecumseh Women

Principal Investigator: Dr. Millicent W. Higgins
Performing Organization: University of Michigan
City and State: Ann Arbor, MI

Contract Number: NO1-CB-84317
Starting Date: 9/30/78 Expiration Date: 3/29/80

Goal: The purpose of the research is to identify precursers of (or risk factors for) breast cancer in women. Cholesterol and triglyceride levels and other known or suspected risk factors will be related to the incidence of breast cancer and of benign breast disease in the female population of Tecumseh, Michigan.

Approach: The population being studied includes all women who were age 20 or older when examined in the Tecumseh Community Health Study. These 3,000 women are currently being followed up as part of an ongoing study on chronic obstructive lung disease or of this study on breast cancer. All cases of breast cancer to date will be identified and the diagnosis of breast cancer confirmed by review of medical and hospital records. It is estimated there will be about 60 cases of breast cancer ascertained. An age-matched control group, three controls per case, will be selected. Additional medical history details on risk factors for breast cancer will be collected for the cases and controls. The entire population of 3,000 women will serve as another comparison group for all items of existing data. The data will be analyzed for possible relationships between cholesterol and triglycerides and breast cancer incidence, with appropriate evaluation of other etiologic factors as well.

Progress: During the six months since the contract was awarded, progress has been made in identifying cases of breast cancer and in analysis of data on benign breast disease. To date, we have 65 potential cases of breast cancer available for study and expect another 25 or so will be identified by the end of June. Medical history forms are being designed to facilitate review of hospital charts for information on the site, type and stage of breast cancer in the cases. Supplemental histories will be obtained from surviving cases and matched controls in the period beginning July 1, 1979. A comparison of women reporting benign breast disease and all other women excluding those with breast cancer has begun. Information collected between 1962 and 1965 is being used. There were 193 cases, of whom 137 said they had had benign breast tumors, 39 reported cystic mastitis or chronic cystic conditions of the breast, and 17 reported both conditions. The comparison group consisted of 2,669 women. Age-specific comparisons have been made of a variety of demographic, physical, and physiological characteristics as well as medical, reproductive, and dietary history.

Project Officers: Elizabeth P. Anderson, Ph.D., Helen Hubert, Ph.D.
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Committee

FY 79 Funds: $66,250

CONTRACT RESEARCH SUMMARY

Title: Benign and Noninvasive Breast Lesions in Groups at Different Breast Cancer Risk

Principal Investigator: Dr. Sue Amelia Bartow
Performing Organization: University of New Mexico
City and State: Albuquerque, NM

Contract Number: NO1-CB-84231
Starting Date: 9/30/78 Expiration Date: 9/29/79

Goal: To investigate whether population groups with low incidence of breast cancer also have a low prevalence at autopsy of benign breast lesions.

Approach: Combining the resources of the population-based New Mexico Tumor Registry, the State's Medical Investigator's Office and the Medical School Department of Pathology, we propose to examine and compare benign breast lesions in three ethnic groups in an autopsy series--Anglos, Spanish Americans, and American Indians. These three groups are all at differing risk for breast cancer, and the incidence rates and histopathologic distributions of breast cancers are already known from Tumor Registry data. The autopsy series to be examined will include about 500 women in three years, 230 Anglo, 140 Spanish American, and 130 American Indian. Breast lesions will be classified pathologically. Radiological studies of the specimens by both clinical mammography and high resolution techniques will be compared with histopathological findings. Medical, reproductive, and related history of each case will be obtained from the family if at all possible.

Progress: Procedures have been tested and established for the multiple facets of the study: (1) specimen collection and preservation, (2) specimen radiography, (3) pathology, gross and micro, (4) demographic data, (5) interview data, (6) record keeping. This has included development of forms and log sheets, design and testing of the medical history (interview) form, and hiring and training of technicians. Specimen collection began December 1, 1978. Both manual files of materials and data and computerized data files are being maintained. Forty cases have been accessioned thus far (27 Anglo, 6 Indian, 7 Spanish). The rate of case acquisition in the first five complete months of collection (December-April) has been less than projected, but it is increasing and we still expect to accession 500 cases in the three-year period. Dr. Malcolm Pike's epidemiologic and statistical participation in the study has been very extensive and very useful, and at less cost than projected. He has made two trips to Albuquerque (one at no expense to our study), and he has been in frequent contact by mail and phone as specific issues arise.

Project Officers: Elizabeth P. Anderson, Ph.D., Louise Brinton
Program: Breast Cancer Epidemiology
Technical Review Group: Breast Cancer Task Force Committee

FY 79 Funds: $87,200

CONTRACT RESEARCH SUMMARY

Title: Relationship of Thyroid Function to Growth of Mammary Tumors

Principal Investigator: Dr. A. David Goodman
Performing Organization: Albany Medical College
City and State: Albany, NY

Contract Number: NO1-CB-74107
Starting Date: 8/15/77 Expiration Date: 8/14/79

Goal: To determine whether hypothyroidism is a significant risk factor for breast cancer.

Approach: DMBA will be administered intragastrically or intravenously, or directly applied to inguinal mammary glands in Sprague-Dawley rats, before or shortly after induction of hypothyroidism by iodine deprivation or propylthiouracil administration, to study the effect of hypothyroidism on the initiation and growth of DMBA-induced mammary cancers. Estrogen dependence and prolactin binding of the DMBA-induced tumors will be examined in hypothyroid and euthyroid animals. The effect of TSH on the DMBA-induced cancer also will be studied.

Progress: Severe hypothyroidism, produced by placing propylthiouracil (PTU), 4 mg %, in the drinking water, caused marked impairment of weight gain and a marked decrease in the incidence of DMBA tumors. When rats receiving 4 mg % PTU were also given T_4 and T_3 to prevent hypothyroidism, the weight gain was normal and the incidence of DMBA-induced tumors was the same as in the control rats. Administration of 0.7 mg % PTU, which causes mild hypothyroidism, was begun either 14 days before or 4 days after administration of DMBA and was continued for 4 months. In the rats made hypothyroid after administration of DMBA, there was a significant decrease in the incidence of tumors, whereas the rats made hypothyroid prior to the administration of DMBA did not have a decrease in tumor incidence compared to the euthyroid group. This raises the possibility that the existence of hypothyroidism prior to administration of DMBA enhanced the induction of mammary cancer by DMBA, which offset an inhibitory effect of hypothyroidism on the subsequent growth of the tumors. A study is in progress to investigate this possibility. In a separate study it has been found that DMBA tumors developing in hypothyroid rats are as likely to regress following ovariectomy as are tumors developing in euthyroid animals.

Project Officer: Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: 0 Terminal

CONTRACT RESEARCH SUMMARY

Title: Effects of Nucleic Acids on the Biological Properties of Mammary Cancer

Principal Investigators: Drs. Janet S. Butel and Florence Farber
Performing Organization: Baylor College of Medicine
City and State: Houston, TX

Contract Number: NO1-CB-53904
Starting Date: 4/1/75 Expiration Date: 3/31/79

Goal: To determine the relationship between transfer of nucleic acid preparations into cells and possible changes in their growth potential.

Approach: The approach has been four-fold: (1) To establish well-characterized mouse mammary tumor cell lines in culture, (2) to determine growth parameters of the tumor cells to monitor in vitro, (3) to characterize and optimize conditions for uptake of exogenous DNA by mammalian cells, and (4) to determine the effect of exogenous DNA on the growth properties of mammary tumor cells.

Progress: Clonal cell lines were established in tissue culture from mammary tumors which arose in BALB/c mice either spontaneously or after treatment with a hormone or a chemical carcinogen. There was no single growth property in vitro which correlated with transplantability in vivo. The ability of the cells to multinucleate in the presence of cytochalasin B did appear to be an indicator of transplantability with the mammary tumor cell lines. Several, but not all, of the transplantable clonal cell lines contained detectable levels of mouse mammary tumor virus (MMTV) RNA. Therefore, MMTV transcription does not appear to be required for maintenance of all mammary gland transformations in mice. The level of MMTV induction by glucocorticoids was only two-fold in the BALB/c tumor cells. Treatment with normal cell DNA did not have any reproducible effects on selected growth properties of two clonal mammary tumor cell lines.

Project Officers: D. Jane Taylor, Ph.D., Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: 0 Terminal

CONTRACT RESEARCH SUMMARY

Title: Effects of Neonatal Exposure to Estrogen and Progestin on the Mammary Gland

Principal Investigator:	Dr. Jeffrey M. Rosen
Performing Organization:	Baylor College of Medicine
City and State:	Houston, TX
Contract Number:	NO1-CB-74189
Starting Date:	8/29/77
Expiration Date:	8/28/80

Goal: To study the influence of perinatal estrogen and progesterone administration on the development and expression of differentiated function in the murine mammary gland.

Approach: Newborn BALB/c mice will be injected with estrogen or progesterone or with both hormones. The organ cultured mammary glands will be exposed to: (a) insulin, hydrocortisone, and prolactin; (b) carcinogenic and non-carcinogenic analogues of DMBA; and (c) thyroxine and growth hormone. Perinatal hormone treatment in virus-positive and -negative BALB/c strains of mice will be compared. In addition, the effect of pretreatment with estradiol and progesterone for either 6 or 9 days at 4 or 5 weeks of age prior to organ culture will be compared. Morphological and biochemical analyses including determinations of casein mRNA and casein will be done.

Progress: Altered morphologic responses were observed after culture in tissue of BALB/c mice injected as newborns with estradiol or with estradiol and progesterone. Alveolar and lobular responses as well as induction of casein mRNA were enhanced as the duration of pretreatment was increased from 6 to 9 days. Minimal development occurred after culture with insulin alone. Culture with cortisol, prolactin, and insulin in combination resulted in maximal alveolar and lobular development. In agreement with morphological data, perinatal estrogen administration resulted in an enhanced induction of casein mRNA in the presence of insulin, cortisol, and prolactin, while perinatal progesterone treatment inhibited casein mRNA accumulation compared to untreated controls. Perinatal estrogen plus progesterone was without morphologic or biochemical effect on the parameters measured. A comparison of the prolactin dose-response in vitro suggested that exposure to estrogen perinatally sensitized the mammary tissue to the subsequent addition of prolactin in culture. Patterns of casein synthesis within mammary tissue sections are being evaluated using a highly specific fluorescent antibody technique.

These results indicate that perinatal estrogen may enhance hormone-regulated mammary differentiation; that perinatal progesterone may inhibit later development; and that both steroids in combination may exert antagonistic effects, perhaps by direct action at the tissue level.

Project Officer: Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $79,590 Terminal

CONTRACT RESEARCH SUMMARY

Title: Prevent the Formation and Progression of Mammary Gland Preneoplastic Lesions

Principal Investigator: Dr. Richard C. Moon
Performing Organization: IIT Research Institute
City and State: Chicago, IL

Contract Number: NO1-CB-74207
Starting Date: 8/29/77 Expiration Date: 8/28/80

Goal: To study the use of retinoid to prevent the development and/or progression of preneoplastic lesions of the mammary gland.

Approach: Non-toxic doses of synthetic analogues of vitamin A (retinoid) will be administered to experimental animals in the diet to study the effect on incidence of HAN and spontaneous mammary tumors in C3H/He mice and DMBA-induced hyperplasias and tumors in BALB/c mice. The prevention of the progression of these ductal lesions and DMBA-induced mammary tumors is planned. Similar studies are planned using the DMBA- and/or MNU-induced lesions in the Sprague-Dawley rat. The effect of retinoids on carcinogen uptake and binding and the effect of time of initiation of retinoid treatment on DMBA- or MNU-induced carcinogenesis in the Sprague-Dawley rat will be examined. Studies will be designed to investigate the mechanisms by which retinoids are effective.

Progress: Studies are in progress to determine the effect of retinyl acetate (RA), axerophthene (AX), retinyl butyl ether (RBE), retinyl methyl ether (RME), and 4-hydroxyphenyl retinamide (4-HPR) on the incidence of HAN and mammary tumors in C3H/He mice and on DMBA-induced hyperplasia and tumors in BALB/c mice. In C3H/He mice, all retinoids except RBE decreased ductal branching and end bud proliferation. In BALB/c mice, RA, RME, and 4-HPR caused a dilatation of ducts, a reduction in the extent of ductal growth and branching, and a flattening and loss of ductal cellularity. In rats, feeding of retinoid prior to administration of carcinogen afforded greater anti-cancer activity than was provided by initiation of retinoid treatment during the progression stage of carcinogenesis. Retinoid also inhibited the development of ovarian hormone-independent mammary tumors. ^{3}H-retinoic acid (all trans) binding proteins (RABP) were separated as 2S components using sucrose density gradient analysis. Carcinogen-induced tumors bound both retinoic acid and retinol specifically. Retinyl acetate and 4-hydroxyphenyl retinamide, which are effective against mammary carcinogenesis, competed for the retinoic acid binding sites only moderately. Increased levels of RABP were found in the ovarian hormone-independent tumors. MNU did not induce retinoid receptors. Human breast cancers contain retinoid receptors. Current studies are evaluating whether a relationship exists between steroid and retinoid receptor levels.

Project Officer: Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $90,300 Terminal

CONTRACT RESEARCH SUMMARY

Title: The Response of the Embryonic Mammary Gland to Androgenic Hormones

Principal Investigator: Dr. Klaus Kratochwil
Performing Organization: Institut für Molekularbiologie
City and State: Salzburg, Austria

Contract Number: NO1-CB-33883
Starting Date: 6/29/73 Expiration Date: 3/28/80

Goal: To study the acquisition of hormone responsiveness during development of the mammary gland. Analysis of the androgen response with special emphasis on the role of tissue interaction.

Approach: Determine androgen-sensitive phase of mammary rudiment by exposing explanted mammary buds of various developmental stages to testosterone in vitro. Determine the target tissue for the hormone by experimental tissue combinations (gland epithelium and surrounding mesenchyme) taking advantage of the androgen-insensitive Tfm-mutant. Find out how the primary target tissue affects its partner (methods: experimental tissue combinations, electron microscopic and histochemical study of the response itself, autoradiography of suitable precursors, especially of tissue interface material). Determine whether the duration of the androgen-sensitive phase of the gland is specified by transient hormone responsiveness of the (primary) target tissue or by changing properties of its partner (hormone binding in relation to responsive phase, combination of tissues in different developmental stages). Compare androgen response in different mammalian species.

Progress: Established that testosterone response in vitro is identical to the reaction in vivo. The temporary androgen-sensitive phase in mammary gland development was found to reflect intrinsic developmental changes in the gland itself. Established that the target tissue for testosterone is the mesenchyme. Found that several steps of cell and tissue interactions are required before and in the androgen response. At least 2 days before the hormone response mammary epithelium has to act on neighboring mesenchymal cells. This epithelial influence is organ- but not species-specific. Later, the condensation of the mesenchyme can be initiated only by cells at the epithelio-mesenchymal interface from where the reaction spreads to more distant cells. Finally, this testosterone-activated mesenchyme (but not the hormone itself) causes necrosis of the epithelium. Although it was found that this action of the mesenchyme on mammary epithelium involves degradation of the epithelial basal lamina, the mechanism of the "mesenchymal attack" is not known. The response of rat glands resembles that of mouse glands, whereas rabbit mammary rudiments are unresponsive to testosterone.

Project Officers: Chester V. Piczak, B.S., E. Brad Thompson, M.D.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $60,600

CONTRACT RESEARCH SUMMARY

Title: Influence of Mammary Carcinoma Cells on the Formation and Absorption of Collagen

Principal Investigator: Dr. Gerhard Wirl
Performing Organization: Institut für Molekularbiologie
City and State: Salzburg, Austria

Contract Number: NO1-CB-74109
Starting Date: 9/1/77 Expiration Date: 8/14/80

Goal: Studies are designed to: (a) extract collagenase directly from DMBA-induced tumors in the rat and spontaneous tumors in C3H mice as well as from tumor tissue cultured media; (b) purify the enzyme and examine its purity using disc gel electrophoresis and immunodiffusion; (c) detect the activity of the enzyme; (d) determine the distribution of the enzyme in the tumor; (d) find any inactive form of the enzyme as a proenzyme; (f) determine the production of collagenase by epithelial cells or stromal cells. Studies of human tissues depends on results obtained from the mouse and rat.

Progress: About one third from a total of 65 DMBA-induced tumors of the rat was found to contain collagenase in great amounts. The same tumors also produce collagenase in vitro. Cultures of small tissue fragments on steel grids release collagenase into the medium and exhibit collagenolytic activity when grown on collagen gels. The cell type responsible for the collagenase release was investigated. Using a method developed by Dr. R. Hallowes, Imperial Cancer Research Foundation Laboratories, London, England, to dissociate and fractionate DMBA-induced tumors, epithelial cell clumps (organoids) and a stromal fraction were obtained. Macrophages attached to the organoids or contaminated the stromal fraction depending on the dissociation procedure. Only media from epithelial and stromal cell cultures contaminated to a high degree with macrophages contain collagenase in an active and/or latent form that is trypsin activated. These results suggest that the macrophage is most responsible for the production of collagenase in vitro.

In contrast to the mammary tumors of the C3H mice, collagenase could be detected in vivo in the Walker 256 tumor of the rat. Other studies indicate that collagenase is present apparently in a latent form and that activation occurs via limited proteolysis by serine proteases.

Project Officer: Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $48,500 Terminal

CONTRACT RESEARCH SUMMARY

Title: Animal and Human Mammary Tumors and Human Cell Culture Bank Facility

Principal Investigator: Dr. Arthur E. Bogden
Performing Organization: Mason Research Institute
City and State: Worcester, MA

Contract Number: NO1-CB-74175
Starting Date: 6/28/63 Expiration Date: 6/27/80

Goal: To function as a service facility for the cryopreservation, storage, and distribution of biologically characterized and monitored (a) animal and human mammary and endocrine-related tumors transplantable in vivo, and (b) cell lines of human and animal breast tumor origin established in in vitro culture, for use by the BCTF and other selected investigators.

Approach: Cryopreservation of mammary and endocrine-related tumors of animal and human origin, as well as human breast tumor cell lines: (a) submittal of tumors and cell lines by qualified investigators able to furnish pertinent background information; (b) biological characterization of transplantable tissues, both pre- and post-cryopreservation, by determination of growth curves, specific effects on host organs, host (syngeneic and xenogeneic) survival, serum hormone levels, histology, karyology, response to ablative procedures; (c) cell lines tested for viability, plating efficiency, freedom from contamination, and tumorigenicity by nude mouse implantation; and (d) characterized tissue and cell lines preserved in Linde liquid nitrogen freezers according to well-recognized, proven procedures.

Progress: During the past contract period, 174 tumors have been shipped to 81 investigators; 49% were of mammary and 15% of anterior pituitary origin. There were 135 new tumors received for cryopreservation, resulting in an inventory of 312 different tumors or sublines stored in 5,516 ampules in liquid nitrogen. Thirty tumors were carried in serial transplantation for characterization studies and 22 for replenishment of Tumor Bank inventory. There are 16 human mammary cell lines as well as the WI-38 and 13762 rat mammary ascites tumor lines cryopreserved, characterized, and free of mycoplasma. Cooperative effort with Dr. W. L. McGuire permitted establishing hormone receptor profiles on 8 mammary cell lines. Fifty-eight shipments, which include 318 viable cultures, 69 frozen ampules and/or gram quantities of packed cells, have been made to 30 different laboratories. Six human mammary tumors, transplantable in athymic mice, are now cryopreserved and available for distribution. Human and rat α-lactalbumin and specific antisera prepared by Dr. Kurt Ebner are also being distributed. A bibliography relative to transplantable tumors in the bank has been compiled and is available on request.

Project Officers: Chester V. Piczak, B.S., D. Jane Taylor, Ph.D.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $169,870

CONTRACT RESEARCH SUMMARY

Title: Identification of Mammary Tissues

Principal Investigator:	Dr. Melvyn S. Soloff
Performing Organization:	Medical College of Ohio
City and State:	Toledo, OH

Contract Number: NO1-CB-63983
Starting Date: 6/30/76 Expiration Date: 6/29/80

Goal: To isolate, identify, characterize, and quantitate myoepithelial cells in rat mammary tissue and tumors of rat and human origin.

Approach: Separate myoepithelial cells by centrifugation in albumin gradients, using oxytocin binding, and scanning/transmission electron microscopy to identify the cells. Examine the potential of using alkaline phosphatase and ATPase as cell markers. Prepare specific antiserum and identify, quantitate, and characterize myoepithelial cells in mammary glands from virgin, pregnant, and postpartum rats and in rat mammary tumors induced with DMBA. Parallel investigations will be carried out with human tumors obtained from surgery.

Progress: Isolated myoepithelial and secretory cells have been purified from the mammary gland of the lactating rat. The two cell types could be distinguished morphologically with the optical, transmission electron, and scanning electron microscope. Myoepithelial cells were stained cytochemically for alkaline phosphatase activity. Isolated myoepithelial cells possess at least 10 times more p-nitrophenol phosphatase activity, Na, K-ATPase activity and high affinity sites for [^{3}H]oxytocin than secretory cells. The secretory cells contained glucose-6-phosphate dehydrogenase activity, which was not detectable in myoepithelial cells. Specific antisera to myoepithelial cells have been produced in rabbits and can be demonstrated by peroxidase-antiperoxidase staining of frozen sections of rat mammary tissues and isolated cells. [^{3}H]oxytocin binding and alkaline phosphatase activities were studied as markers for mammary myoepithelial cells from the onset of pregnancy, through parturition and in lactation. Both activities were lowest at the beginning of pregnancy, increased steadily during pregnancy, and were maximal during lactation. Twenty-seven human breast samples have been assayed for [^{3}H]oxytocin binding activity. The binding was more substantial in fibroadenomas and in some samples from patients with fibrocystic disease. Binding was negligible in adenocarcinomas of the breast. These findings are consistent with the impression that myoepithelial cells may be involved in the pathogenesis of sclerosing adenosis and they may become prominent in fibroadenomas.

Project Officers: Chester V. Piczak, B.S., William Kidwell, Ph.D.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $78,600 Terminal

CONTRACT RESEARCH SUMMARY

Title: Prevent the Formation and Progression of Mammary Gland Preneoplastic Lesions

Principal Investigator: Dr. Clifford W. Welsch
Performing Organization: Michigan State University
City and State: East Lansing, MI

Contract Number: NO1-CB-74090
Starting Date: 8/29/77 Expiration Date: 8/28/80

Goal: To prevent the early development of preneoplastic mammary gland lesions and progression of these preneoplastic lesions to neoplastic lesions by chronic suppression of pituitary prolactin secretion.

Approach: A prolactin-suppressing drug, 2-bromo-α -ergocryptine (CB-154), will be administered prior to and after the spontaneous development of, or the induction of, mammary gland dysplasias. The following mammary gland dysplasias will be examined: A. Mice: (1) spontaneously occurring alveolar lesions (HAN) - DBA/2; (2) pregnancy-induced alveolar lesions (HAN) - A/J; (3) DMBA-induced lesions (DH) - BALB/c; (4) estrogen- and progesterone-induced and pregnancy-induced ductal lesions (plaques) - GR/A. B. Rats: (1) DMBA-induced ductal and alveolar lesions - Lewis and Sprague-Dawley; (2) X-irradiation-induced ductal and alveolar lesions - Sprague-Dawley; (3) NMU-induced ductal and alveolar lesions - Sprague-Dawley. C. Retinyl acetate feeding will be compared with prolactin suppression on the genesis of mammary dysplasias in NMU-treated Sprague-Dawley rats and estrogen-progesterone-treated GR/A female mice.

Progress: The suppression of prolactin secretion with CB-154 has markedly reduced the incidence of mammary gland dysplasias in carcinogen (DMBA and NMU)-treated female Sprague-Dawley rats and carcinogen (DMBA)-treated female Lewis rats. Suppression of prolactin secretion has also sharply reduced the incidence of pregnancy- and estrogen-progesterone-induced mammary gland dysplasias in female GR/A mice. Suppression of prolactin secretion does not influence the development of mammary gland dysplasias in carcinogen-treated female BALB/c mice. Prolactin suppression appears to be just as effective as retinyl acetate feeding in the prophylaxis of NMU-induced mammary tumorigenesis in female Sprague-Dawley rats. Prolactin suppression reduces the incidence of irradiation-induced mammary tumorigenesis in female Sprague-Dawley rats. It also appears that the dose of the carcinogen markedly influences the effectiveness of prolactin suppression, i.e., in low doses of administered carcinogens (DMBA), prolactin suppression appears to be far more effective in prophylaxis of murine mammary tumorigenesis.

Project Officer: Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $80,200 Terminal

CONTRACT RESEARCH SUMMARY

Title: Metabolism of Polycyclic Aromatic Hydrocarbons in the Induction of Mammary Tumor

Principal Investigator: Dr. A. Monaem El-hawari
Performing Organization: Midwest Research Institute
City and State: Kansas City, MO

Contract Number: NO1-CB-84227
Starting Date: 9/1/78 Expiration Date: 8/31/80

Goal: To determine whether the disposition, metabolism, and binding are responsible for differences in mammary tumor incidence casued by polycyclic aromatic hydrocarbons.

Approach: Experiments will be done on the following eight groups of rats: mature virgin females from Sprague-Dawley, Long Evans, and Lewis strains; mature male rats from Sprague-Dawley strain; young, old, pregnant, and lactating rats from Sprague-Dawley strain. Tumors will be induced with either 3-MC (66 mg/100 g) or DMBA (13.3 mg/100 g). Control animals will receive sesame oil (1 ml/100 g). Disposition and metabolism studies will be done both in vivo and in vitro. Covalent binding to macromolecules and various enzyme activities will be determined.

Progress: Tumor induction studies confirmed the high susceptibility of virgin female Sprague-Dawley rats to mammary tumor induction by DMBA. Tumors were induced in Wistar Lewis rats after a longer latent period while Long Evans rats showed low susceptibility. Similar responses were demonstrated after treatment with 3-MC, but the carcinogenic indices were lower. Sexual maturity of rats was confirmed before dosing. Disposition studies revealed no major differences in absorption or elimination of DMBA in the three strains of rats. However, levels of MC and/or its metabolites disappeared less readily from breast tissues of Sprague-Dawley rats. Metabolic profiles in tissues and excreta are being analyzed for qualitative differences. A few hours after administering DMBA or 3-MC to pregnant rats, the carcinogens and their metabolites were found in fetal tissues. This, however, did not seem to alter the disposition of the carcinogens. While concentrations in maternal tissues declined, those in fetuses remained high. Both carcinogens were also transferred to the newborns through their mothers' milk. In vitro, both carcinogens were metabolized by the mammary gland. DMBA metabolites seemed to differ from those produced by the liver. Methods are under development for metabolic studies in intact cells from liver and mammary gland.

Project Officer: Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $155,500

CONTRACT RESEARCH SUMMARY

Title: Produce and Identify Antibodies to Collagens/Procollagens and/or Related Enzymes

Principal Investigator:	Dr. Burton Goldberg
Performing Organization:	New York University Medical Center
City and State:	New York, NY
Contract Number: NO1-CB-84314	
Starting Date: 9/1/78	Expiration Date: 8/31/80

Goal: To produce specific antibodies to the carboxyterminal non-helical sequences of human type I procollagen in sufficient quantities for storage and distribution to other investigators.

Approach: Culture human skin fibroblasts (CRL1121) in roller bottles and isolate the procollagen in the culture medium, then subject it to specific bacterial collagenase digestion. Purify the carboxyterminal fragment by ion exchange chromatography, then produce antibodies in appropriate animal species (rabbit, goat, sheep). Purify the antisera by absorption techniques and/or affinity chromatography and confirm their specificity by Ouchterlony technique, immunoelectrophoresis, passive hemagglutination, and radioimmunoassay.

Progress: Progress was hampered during the first five months of this project due to the lack of delivery of the roll-in incubator and cell transfer hood by the manufacturer. Consequently, large-scale collection of tissue culture media from roller bottles was delayed. However, during the initial six months of the contract existing tissue culture facilities were used to collect small amounts of the antigen for pilot studies on purification and immunization. The carboxymethyl cellulose ion exchange procedure for purification of the antigen has been modified with respect to pH, urea concentration, and salt elution to effect better recoveries of the protein. Rabbits have been immunized with the antigen and 200 ml of sera have been collected which have an average titer of 1/1600 in a specific radioimmunoassay.

For the remaining six months of the contract year it is planned (1) to purify sufficient amounts of the antigen (6-12 mg) from roller bottle collections to complete an immunization schedule in a sheep or goat, and (2) to develop an affinity chromatography procedure for purification of monospecific antibodies. The available rabbit antiserum will be used for pilot studies and the sheep or goat antisera will be utilized as they become available.

Project Officer: Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $68,200

CONTRACT RESEARCH SUMMARY

Title: Development of an Assay for Genetic Damage to Mammary Gland Cells

Principal Investigator: Dr. Ronald W. Hart
Performing Organization: Ohio State University
City and State: Columbus, OH

Contract Number: NO1-CB-84226
Starting Date: 9/1/78 Expiration Date: 8/31/80

Goal: To develop an inexpensive, rapid, and quantitative assay for detecting genetic damage and its repair in mammary cells in vivo following exposure to carcinogenic agents.

Approach: Measure DNA damage directly by an assay using S_1 endonuclease. Determine changes in the size of non-radiolabeled DNA resulting from endonuclease treatment using alkaline or neutral sucrose gradient sedimentation. After sedimentation, the non-radiolabeled DNA will be quantified by a spectrofluorometric technique. Quantitate the mammary epithelial cell DNA damage by double label autoradiography. DMBA-sensitive and -resistant rat strains and non-carcinogenic and less carcinogenic analogs 2-FL-DMBA and 5-FL-DMBA will be used for these studies.

Progress: Since the inception of this contract, surgical procedures were developed that allow removal of a complete set of mammary glands for assay while leaving the other set intact within the host for scoring tumor incidence. Procedures for isolation of nuclei were developed to obtain approximately 75,000 nuclei per gland, free of adipose and connective tissue, for break and S_1 endonuclease assays. Developed techniques for isolation of mammary gland DNA free of collagen and fat for determining the total amount of radioactivity bound to DNA. HPLC pure 2-fluoro-DMBA was synthesized and custom labeled ^{3}H-2-fluoro-DMBA was obtained. Optimal conditions for recognition of DMBA-induced damage by S_1 endonuclease were determined. An alkaline agarose gel electrophoresis system was developed to compare the molecular weight of mammary DNA from 16 different samples simultaneously. Sephadex LH-20 and high pressure liquid chromatography (HPLC) techniques were developed for analyzing the DN-HC products formed. Cold and H-labeled DMBA and 5-fluoro-DMBA, which are necessary to perform future experiments, were synthesized and/or purchased.

Project Officer: Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $127,800

CONTRACT RESEARCH SUMMARY

Title: Glycoproteins of the Mammary Cell Surface

Principal Investigator: Dr. Eugene A. Davidson
Performing Organization: Pennsylvania State University
City and State: Hershey, PA

Contract Number: NO1-CB-63984
Starting Date: 6/20/76 Expiration Date: 6/18/79

Goal: To isolate and characterize the cell surface glycoproteins and other complex carbohydrates produced by human mammary carcinoma cells.

Approach: Apply techniques for surface labeling of the protein and carbohydrate moieties of the glycoproteins on the cell plasma membrane. Assess the labeled macromolecules in terms of molecular size and localization within the membrane. Apply the best method for isolation and purification of plasma membranes. Characterize the purified plasma membranes for their chemical architecture, biological and immunological properties, and enzyme markers. Human mammary cell lines obtained from the BCTF Cell Bank will be used for the studies.

Progress: The elucidation of the structure of the major oligosaccharide chain of the mucin-type glycoproteins of the MCF-7 and MDA-MB-231 cells has been accomplished by in vivo labeling of the sugar moieties by cultivating cells in medium containing ^{3}H-glucosamine and ^{14}C-galactose and sequential degradation of the oligosaccharide chain with specific glycosidases. Both gel filtration and paper chromatography have been utilized to identify the products at each stage of glycosidase treatment. The oligosaccharide structure thus arrived at is Galβ→GlcNAcβ→Galβ→GalNAc, which is strikingly similar to the oligosaccharide structure reported for the murine mammary tumor glycoprotein, epiglycanin. Further, it has been well established that this is the only major oligosaccharide unit of the mucin type glycopeptides of the cancer lines MDA-MB-231 and MCF-7.

The investigation on the nature of glycosaminoglycans (GAGs) produced by these cell lines has been completed. In addition to the sharp differences noticed in the pattern of GAGs between control and tumor lines, this extensive study has brought forth several valuable findings including (a) the heparan sulfate associated with the cells is quite large in molecular size and (b) the occurrence of some hyaluronidase-susceptible linkages in heparan sulfate and heparitinase-susceptible linkages in chondroitin sulfate.

The glycoprotein fraction has been purified by DEAE-cellulose chromatography and affinity chromatography (anti-fetal calf serum IgG-Sepharose and wheat germ agglutinin-Sepharose). Antiserum for that glycoprotein is now being raised in rabbits.

Project Officers: Chester V. Piczak, B.S., D. Jane Taylor, Ph.D.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: 0 Terminal

CONTRACT RESEARCH SUMMARY

Title: Change in Mammary Sensitivity to Carcinogens Related to Host Physiological State

Principal Investigator: Dr. Dilip K. Sinha
Performing Organization: Roswell Park Memorial Institute
City and State: Buffalo, NY

Contract Number: NO1-CB-74105
Starting Date: 8/29/77 Expiration Date: 8/28/80

Goal: To investigate the susceptibility of the mammary gland to neoplastic transformation during changing conditions of the host due to age and pregnancy.

Approach: Study the DNA synthesis of ductal and alveolar cells of prepubertal, pubertal, and postpubertal rats. Attempt to induce growth in mitotically static old mammary glands by transplantation. Determine the proliferative pattern of "old" and "young" mammary glands transplanted into the gland-free mammary fat pad. Attempt to induce tumors in old mammary glands transplanted into the gland-free fat pads of old rats.

Progress: The growth and development of the mammary gland in prepubertal, pubertal, and postpubertal (aging) rats have been studied. Ten-, 15-, 25-, 35-, 55-, 70-, 90-, 150- and 250-day-old female rats were used in this investigation. The developmental pattern is established by studying whole-mount preparations of the abdomino-inguinal gland and by DNA synthetic index as indicated by ^{3}H-thymidine uptake (labeling index). Mammary glands in 10-day-old rats start as a single, branched duct with club-shaped end buds, which with age develop into multiple ductal system and ultimately almost fill up the fat pad. The proliferative index reaches its peak in 25 and 35 days; it then starts to decline. At 150 days and after, the glands are almost at stasis, considering proliferation. The second approach of this project was to investigate the proliferation and growth of pieces of mammary glands transplanted into isologous hosts. Small pieces of mammary tissue from 60-day-old rats were transplanted into the gland-free fat pad of 200-day-old rats and vice versa. Graft-bearing rats were killed at 60 days, 90 days, 120 days, and 150 days after transplantation. Also, dissociated mammary cells from 200-day-old female rats were injected into the fat pads of isologous hosts. Both the small pieces and the dissociated cells grew to form a full ductal mammary structure. ^{3}H-thymidine labeling indices of these outgrowths were comparable to mammary glands from 50-60-day-old virgin female rats. Tumorigenesis in these outgrowths by 7,12-DMBA is being tested. Experiments are in progress and no meaningful data were available at the time of this report.

Project Officer: Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $59,700 Terminal

CONTRACT RESEARCH SUMMARY

Title: Mammary Gland Responsiveness to Multiple Hormones

Principal Investigators:	Drs. W. B. Dandliker and R. J. Brawn
Performing Organization:	Scripps Clinic and Research Foundation
City and State:	La Jolla, CA

Contract Number: NO1-CB-43905
Starting Date: 6/1/74 Expiration Date: 6/30/79

Goal: Determine mechanism(s) underlying the ability of the mammary gland to respond to several steroid and peptide hormones. Investigate possible competitive, cooperative, or sequential effects in the binding of hormones to their specific cellular receptors of mammary tissue. Based upon these findings, develop simple fluorescence receptor assays useful in the clinical laboratory.

Approach: Fluorescent tagging of hormones, especially the steroid hormones, purification of the fluorescent derivatives by chromatography, and testing of their binding activity by the dextran-coated charcoal method, by fluorescence polarization and by fluorescence microscropy. Specificity in each case is defined by the ability of the fluorescent labeled hormone to compete with estradiol in binding to receptor preparations, cells, or tissue sections from mammary tumors as compared to normal controls from hormone-responsive or non-responsive tissues.

Progress: The properties and use of an estrogen conjugated to fluorescein have been extensively investigated (Dandliker et al., Cancer Research, in press; Rao et al., International Congress on Hormonal Steroids, New Delhi, October 1978). This conjugate, 17-Fε, is applicable to rapid receptor assays in cytosol preparations and selectively stains only certain cells as viewed in the fluorescence microscope; in normal uterine tissue only epithelial cells are stained while in certain breast carcinomas only the malignant ductoid elements are stained. Pursuing the leads offered by 17-Fε has led to the synthesis of even more highly specific fluorescent conjugates derived from the highly active estrogen, 17α-ethynyl estradiol. The methodology for applying fluorescent-labeled hormones to receptor studies is now in print (Dandliker et al., Methods in Enzymology, 48F:380-415, 1978).

Project Officers: Chester V. Piczak, B.S., E. Brad Thompson, M.D.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: 0 Terminal

CONTRACT RESEARCH SUMMARY

Title: Role of Stroma in Growth of Neoplasms of the Mammary Gland

Principal Investigator: Dr. Merton R. Bernfield
Performing Organization: Stanford University
City and State: Stanford, CA

Contract Number: NO1-CB-53903
Starting Date: 4/15/75 Expiration Date: 4/14/80

Goal: To define the role of the basal lamina, its constituent glycosaminoglycan (GAG), and the stroma in postnatal mammary development and neoplasia.

Approach: Elucidate the mechanisms underlying and controlling the assembly, maintenance, and loss of the basal lamina during mammary epithelial morphogenesis and in association with mammary neoplastic invasion.

Progress: Having established that the basal lamina of the postnatal mouse mammary epithelia contains glycosaminoglycan (GAG), the role of GAG synthesis and degradation in the formation of the lamina was examined in secondary cultures of mouse mammary epithelial cells. Cultures on plastic, where no lamina forms, were compared with cultures on type I collagen gels, where a distinct GAG-containing lamina is formed. There is no difference between substrates in the rate of GAG synthesis, but the collagen gel causes a marked reduction in the GAG degradation rate. GAG degradation does not occur in the medium, indicating that degradation is localized to the cells. The cell-associated GAG molecules are in heparan sulfate-rich proteoglycans (K_{av} = 0.35 on Sepharose 4B) which apparently bind to collagen. The GAG molecules in the medium have a similar composition, but the major fraction has a lower apparent molecular weight (K_{av} = 0.53). By autoradiography, the slowly degrading cell-associated pool of GAG present in cultures on collagen is localized near the site of the ultrastructurally identified lamina. Formation of the basal lamina, therefore, may result from collagen-mediated reduction in the degradation of cell surface proteoglycans.

High passage cells of the same origin do not produce an ultrastructurally identifiable basal lamina when cultured on collagen gels. These are neoplastic cells, as indicated in vitro by their continued DNA synthesis at confluence, piling-up, reduced fibronectin, high levels of reverse transcriptase, and in vivo by their ability to produce pleomorphic invasive tumors when injected into nude mice. The rate of GAG production and the types of proteoglycans synthesized by these cells are similar to those of low passage cells, but the GAG degradation rate in high passage cultures is unaffected by collagen. This result strengthens the idea that basal lamina formation results from reduced degradation of proteoglycans and suggests that control of laminar GAG degradation may be altered in neoplastic cells.

Project Officers: Chester V. Piczak, B.S., D. Jane Taylor, Ph.D.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $92,000

CONTRACT RESEARCH SUMMARY

Title: Growth and Passage of Primary Culture of Normal Mammary Epithelial Cells

Principal Investigator: Dr. Dayton S. Misfeldt
Performing Organization: Stanford University
City and State: Stanford, CA

Contract Number: NO1-CB-74094
Starting Date: 7/1/77 Expiration Date: 6/30/80

Goal: To define conditions for the growth and passage of normal mammary epithelial cells in culture.

Approach: Conditions for optimal growth will be determined with cells both from an established mouse mammary line (NMUMG) and from primary cultures of BALB/c mouse and human. To determine optimal growth conditions the following factors will be tested: (a) commercial media; (b) types and concentrations of sera; (c) pH's; (d) culture substrata; and (e) additives to the medium such as hormones, cations, and antibiotics. Various enzymatic and mechanical methods of passaging cells will be tested. The best method(s) of inhibiting fibroblast growth will be determined by adding or deleting certain factors from the growth medium, and physical separation of fibroblasts and epithelial cells.

Progress: The collagenase dissociation of mouse mammary gland gave the highest seeding efficiency. The medium which best promoted growth was high glucose Dulbecco's modified Eagle's medium with 15% fetal calf, bobby calf, or horse serum. The primary cells establish colonies on plastic most rapidly at pH 6.9-7.2. Cultures of primary mammary cells can be obtained as easily from unstimulated virgin mice as from those stimulated with estrogen-deoxycorticosterone.

Cultures of human primary epithelial cells can be obtained from tissue from mammoplasty surgery by digestion in collagenase and plating on plastic by methods similar to those used with mouse tissue.

By plating cells of the established NMUMG line at low densities, they have determined that cloning efficiencies are higher in medium without antibiotics (streptomycin and penicillin). There is a beneficial effect on cloning of pH and the addition of lithium and dithiothreitol to the medium which is being tested.

Medium in which D-valine is substituted for L-valine has been used to inhibit fibroblast overgrowth; however, D-valine medium slows the growth of NMUMG cells to confluency and causes the appearance of many giant cells.

Project Officer: Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $72,800

CANCER RESEARCH SUMMARY

Title: Morphological Properties of Normal and Abnormal Human and Rodent Mammary Tissue

Principal Investigator: Dr. Elinor Spring-Mills
Performing Organization: State University of New York
City and State: Syracuse, NY

Contract Number: N01-CB-84239
Starting Date: 8/14/78 Expiration Date: 8/14/80

Goal: To evaluate structural differences among normal, dysplastic, and cancerous mammary glands.

Approach: To describe, quantitate, and compare certain morphological and functional characteristics of normal, hyperplastic, and carcinomatous human and mouse mammary glands in vivo and in vitro. Human tissues obtained at biopsy, mastectomy, and reduction mammoplasty will be prepared for light microscopy (LM), transmission electron microscopy (TEM), scan electron microscopy (SEM), and quantitative electron microscopy (QEM) or morphometry. Animal tissue from non-pregnant (pubertal and adult), pregnant, and multiparous C3H mice will be studied.

Progress: This period of performance has been devoted primarily to setting up the laboratory and standardizing methods to be used on this project. Additional laboratory equipment is being provided by the university, the lack of which hampered some of the initial investigations. The entire research computer system is being installed and should be operational in July 1979. Endocrine profiles have been obtained on ten women with such lesions as fibroadenomas and infiltrating duct carcinoma using radioimmunoassays. Of particular interest was the observation that patients with fibroadenomas had moderate to pronounced elevations in their prolactin levels. Tumors and hyperplastic lesions from 20 women have been fixed and prepared for transmission and scanning microscopy.

The processing of tumor-free, nodular, and tumor-containing mouse mammary glands is in progress. No results can be reported at this point because photographing of the material has just begun.

Project Officer: Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $76,000

CONTRACT RESEARCH SUMMARY

Title: Study Breast Cancer Transplantability in Nude-Athymic Mice

Principal Investigator: Dr. Beppino C. Giovanella
Performing Organization: Stehlin Foundation
City and State: Houston, TX

Contract Number: NO1-CB-33856
Starting Date: 2/1/73 Expiration Date: 1/31/79

Goal: To ascertain whether a thymusless-nude strain of Swiss mice can be used as transplant-recipient of human tumors for a study of their growth potential, hormone responsiveness, and their response to chemotherapeutic agents.

Approach: A colony of thymusless-nude Swiss mice has been developed, and the good health of the animals permits their use in long-term experiments. Each tumor sample is transplanted into at least 2 adult females and 2 adult males treated with antilymphocyte serum--AL(B)S--subcutaneously. If enough material is available, the tumor is transplanted subcutaneously and intracerebrally in adult untreated nude mice and subcutaneously and intraperitoneally in untreated newborn nude mice with and without a source of angiogenetic factor(s). If the tumor grows, it is retransplanted for at least 3 generations. The transplanted tumors are examined histologically and characterized by a pathologist.

Progress: One hundred ninety-nine primary and metastatic human breast carcinomas and 37 cell lines from human breast cancers have been transplanted in 4,148 nude-thymusless mice. Of 57 breast cancers transplanted directly from the patient into untreated nudes, 28 grew slowly and only 1 or 1.8% could be transplanted serially. Until 2 years ago, of 54 tumors transplanted into AL(B)S-treated mice, 30 grew and 7 or 13% could be transplanted serially. During the past 2 years, using improved preparations of AL(B)S, 22 of 56 breast carcinomas grew, and 9 or 20% could be transplanted serially. The intracerebral route of inoculation is actively investigated. Heterotransplantation of breast carcinomas in untreated newborns and under the kidney capsule of adults has been less successful. Nineteen of 37 cell lines derived from human breast carcinomas grew after subcutaneous injection in untreated adult nudes and two of them produced metastases in the lymph nodes and in the lungs. Five serially transplanted breast carcinomas have been tested for their sensitivity to various anti-cancer drugs in 1,260 nudes. Each tumor exhibited an individual pattern of response to chemotherapy. Two tumor cell lines grew faster in males and two in females.

Project Officers: D. Jane Taylor, Ph.D., Harold Hoffman, Ph.D.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: 0 Terminal

CONTRACT RESEARCH SUMMARY

Title: Influence of Carcinogens of Viral Gene Expression Leading to Mammary Cancer

Principal Investigator:	Dr. Asher Frensdorff
Performing Organization:	Tel Aviv University
City and State:	Tel Aviv, Israel
Contract Number:	NO1-CB-74118
Starting Date:	9/30/77
Expiration Date:	9/29/80

Goal: To study longitudinally the influence of chemical carcinogens alone and in combination with prolonged hormonal stimulation on murine mammary tumor virus-gene expression.

Approach: MuMTV gene expression will be studied by measuring three independent parameters: (1) levels of MuMTV-RNA in mammary gland cells at varying intervals after onset of oncogenic stimuli (post-transcriptional level); (2) levels of MuMTV-antigens expressed on mammary gland tissue and in host serum, milk, or as antigen-antibody complex deposits in the kidneys (post-translational level); (3) autogenous humoral immune response to MuMTV and to MuMTV polypeptides and other antigens. Oncogenic stimuli will be DMBA, NMU, radiation, and prolonged hormonal stimulation. BALB/c mice will be used for these studies.

Progress: MuMTV-RNA in mammary gland cells, expression of MuMTV polypeptides in mammary gland cells, in serum and in milk, as well as the humoral immune response to MuMTV antigens, were the parameters of viral gene expression. These are monitored longitudinally at different times following the oncogenic stimulus. Oncogenic stimuli are DMBA, combined with prolonged hormonal stimulation, irradiation, and superinfection with various MuMTVs. BALB/c mice used in this study are bred in this facility from a very small number of breeding pairs to ensure maximum homogeneity to spontaneous MuMTV gene expression. Groups of mice exposed to different carcinogenic conditions or to a combination of such conditions and factors that affect viral gene expression have now been completed. Most important findings are in hormone- and carcinogen-treated mice. There is a progressive increasing humoral immune response to MuMTV suggestive of MuMTV gene expression (at the polypeptide level) in the host. These findings agree with recent reports in the literature. Experiments are in progress to interfere with this expression, either by passive administration of antiserum to MuMTV or by active immunization with MuMTV. Such experiments will study the relationship between the carcinogenic event and MuMTV expression and even more importantly between MuMTV gene expression and carcinogen-induced mammary tumorigenesis. A better understanding of the mechanism of action of DMBA will be obtained to determine whether or not carcinogen-induced oncogenesis is dependent on viral gene expression.

Project Officer: Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $123,040 Terminal

CONTRACT RESEARCH SUMMARY

Title: Detection and Quantitation of Transformed Mammary Epithelial Cells In Vitro

Principal Investigator: Dr. William Niedermeier
Performing Organization: University of Alabama
City and State: Birmingham, AL

Contract Number: NO1-CB-74092
Starting Date: 8/29/77 Expiration Date: 8/28/80

Goal: To identify and isolate mammary tumor-related cell surface antigens and use these antigens in biochemical assays to discriminate between normal and neoplastic human mammary epithelium.

Approach: Examine human breast adenocarcinomas for the presence of specific biochemical markers. Markers will be identified by immunofluorescence, quantitated by radioimmunoassay, and isolated by immunospecific affinity chromatography. The physicochemical properties including carbohydrate and amino acid compositions will be compared with those of comparable glycoproteins isolated from normal tissues.

Progress: Identified by immunofluorescence, antibodies in blood sera of 45% of patients with adenocarcinoma of the breast reacted with antigenic components in frozen sections of their tumors. Cross reactivities were noted among the patients in whom this reaction was positive. Also, sera from these patients invariably reacted with components in frozen sections of mouse mammary tumors known to contain mouse mammary tumor virus (MMTV). One preparation of rabbit antiserum to MMTV isolated from BALB/cfC3H cells grown in culture reacted with human breast adenocarcinomas. A second preparation of rabbit antiserum to MMTV isolated from milk of tumor-bearing mice failed to react with human adenocarcinomas of the breast, but strong positive immunofluorescence reactions were observed when tested against mouse mammary tumors. Differences in these two antisera and antigens are under investigation.

Tumor cell surface antigens were extracted by mild sonication and fractionated by gel filtration. Active fractions were labeled with ^{125}I and immunoprecipitated with anti-mouse mammary tumor virus or sera from patients with breast adenocarcinoma. Similar experiments were performed using sera from apparently normal females. Variation is observed between individual sera, but the amount of labeled antigen precipitated by sera from patients with adenocarcinoma is 3 to 5 times greater than that precipitated by sera from healthy females. Optimal conditions for immunoprecipitation will be determined to develop quantitative radioimmunoassays for determining circulating antibody and assaying tumor antigen, and to provide purified antigen for physicochemical characterization.

Project Officer: Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $152,400 Terminal

CONTRACT RESEARCH SUMMARY

Title: Microcirculation and Molecular Transport in Mammary Carcinomas

Principal Investigators:	Dr. Joseph F. Gross	Dr. Marcos Intaglietta
Performing Organization:	University of Arizona	University of California
City and State:	Tucson, AZ	San Diego, CA

Contract Number: NO1-CB-63981
Starting Date: 6/14/76 Expiration Date: 6/13/80

Goal: To study local blood velocity and intraluminal hydrostatic pressure in tumor capillaries and quantitate movement of materials across the endothelium of single vessels into extravascular tissue in tumor microcirculation.

Approach: Fit Fischer 344 rats with a modified algire access chamber containing mammary adenocarcinoma for analyses of microcirculatory parameters. Obtain quantitative morphological data by planimetric techniques. Measure velocity of red blood cells with dual slit technique, and capillary hydrostatic pressure with micropipette technique. Use intra- and extraluminal temporal changes in fluorescent marker concentration to determine transport characteristics of endothelial wall and extravascular tissue in tumors. Develop analytical models to characterize microcirculatory transport in tumor systems.

Progress: Minor modifications to the new chamber design ensure a constant volume space for tumor growth. Histological tissue verification of mammary adenocarcinoma preparations was established. The proliferative fraction on the implanted tumor section takes 5-12 days to remove the necrotic section remaining after surgery and another 10 days for the proliferation fraction to be seen. There is a direct inverse correlation between size of donor's tumor and percentage of takes of tumor. Rat BA 1112 sarcoma has been characterized in terms of microvascular parameters, e.g., vessel diameter, red blood cell velocity, and tissue perfusion over 26 days. Inflow through arteriolar vessels increased during the observation period from 0.72 ± 0.08 to 0.95 ± 0.09 mm/sec while red blood cell velocity in post-capillaries and collecting venules decreased slightly, suggesting that venous outflow is facilitated during progressive spread of the tumor. Since vasomotion was only observed during initial phase of tumor growth and arteriolar red blood cell velocity increased during the same period, findings indicate that blood is supplied to mesenchymal tumors by dilated arterioles. Analysis of capillary tissue perfusion demonstrated tissue perfusion gradients between edge of the tumor are between 2-4 ml/min/100 g in the center and between 15-18 ml/min/100 g at the edge despite local and temporal functional inhomogeneity. A similar analysis including microvascular pressure measurements is being performed using DMBA adenocarcinoma in Fischer 344 rats. Data on diffusion of fluorescent materials across the endothelial wall of the tumor blood vessels are in preparation for both tumors. A computer program based on the previous theoretical model for transport in tumor microcirculation has been completed giving 3-dimensional concentration profiles for oxygen distribution.

Project Officers: Chester V. Piczak, B.S., Richard Knazek, M.D.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $84,800 Terminal

CONTRACT RESEARCH SUMMARY

Title: Growth and Passage of Primary Cultures of Normal Mammary Epithelial Cells

Principal Investigator:	Dr. Gordon H. Sato
Performing Organization:	University of California
City and State:	La Jolla, CA
Contract Number:	N01-CB-74188
Starting Date: 8/1/77	Expiration Date: 7/31/80

Goal: To isolate and maintain growth of pure normal mammary epithelial cells in culture.

Approach: Determine the hormonal and nutritional requirements of established animal and human mammary tumor cell lines. Proceed with similar studies on primary cultures of human mammary tumors obtained from autopsies and mastectomies. Determine the hormonal content, blood serum components, and growth factors (ovarian, nerve, epidermal, etc.) to prepare a defined medium to maintain growth and passage of normal mammary epithelial cell lines.

Progress: MCF-7 cells grown in medium containing 10% fetal calf serum (FCS) were detached from flasks with a trypsin-EDTA solution, treated with trypsin inhibitor, and replated in medium in the absence of serum. Medium used for experiments was a 1:1 mixture of Ham's F12 and Dulbecco modified Eagle's medium (DMEM) supplemented with 15 mM HEPES, pH 7.4, 1.2 g/l sodium bicarbonate, 10^{-8} M sodium selenite, and antibiotics. In this medium, cells were stimulated to grow by the addition of insulin (30 to 100 ng/ml), transferrin (25 µg/ml), epidermal growth factor (10 to 100 ng/ml), cold insoluble globulin (7.5 µg/ml), and prostaglandin $F_{2\alpha}$ (10 to 100 ng/ml). There was no growth in the absence of these factors. In a mixture of these factors the generation time of the cells was the same as that of cells in medium with 10% FCS. Growth was better in the F12:DMEM mixture than in either F12 or DMEM alone. Addition of the 5 factors to cells in medium with 10% FCS did not increase cell number beyond that seen with 10% FCS alone. MCF-7 cells have grown continuously for over 2 months in the serum-free medium. Cell attachment and spreading in the serum-free medium was greatly enhanced if an α-1 serum protein was also added. Under these conditions the gross morphology of cells in the defined medium was identical to that of cells in serum. Attempts are being made to adapt the MCF-7 medium to support the growth of epithelial cells in primary cultures from transplantable human breast tumors carried in nude mice. These tumors are estrogen responsive in vivo.

Project Officer: Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $65,300

CONTRACT RESEARCH SUMMARY

Title: Effects of Neonatal Exposure to Estrogen and Progestin on the Mammary Gland

Principal Investigator: Dr. Frank J. Talamantes, Jr.
Performing Organization: University of California
City and State: Santa Cruz, CA

Contract Number: N01-CB-74091
Starting Date: 8/1/77 Expiration Date: 7/31/80

Goal: Determine the effects of perinatal exposure to exogenous estrogens and progestins, singly and in combination, on mammary gland noduligenesis and tumorigenesis in female mice.

Approach: C3H (MTV+, NIV+) and C3Hf (MTV-, NIV+) female mice will be treated perinatally with diethylstilbestrol (DES), 17α-hydroxyprogesterone caproate (HPC), or Ovral. The incidence, types, and age of appearance of mammary gland dysplasias will be recorded to determine whether noduligenesis and tumorigenesis depend on the type and dose of hormone, direct action of hormone on the gland, or on alteration of systemic factors. Changes in mammary sensitivity to an oral contraceptive (Ovral) and a chemical carcinogen (DMBA) after perinatal hormone exposure and the effects of hormone administration to lactating mothers on noduligenesis and tumorigenesis in female offspring will also be determined.

Progress: Prenatal treatment: Primiparous females received daily sc injections of one of the following on days 10-17 of pregnancy: (1) 0.03 µg/day or 0.1 µg/day DES, (2) 15 µg/day or 150 µg/day HPC, (3) 25 ng/day ethinyl estradiol (EE_2) + 250 ng/day norgestrel (N) or 2.5 ng/day EE_2 + 25 ng/day N, or (4) sesame oil. Neonatal treatment: Female offspring of untreated primiparous mothers received daily sc injections of one of the following on days 1-5 of life: (1) 0.001 µg/day, 0.1 µg/day, or 2.5 ng/day DES, (2) 15 µg/day or 150 g/day HPC, (3) 25 ng/day EE_2 + 250 ng/day N or 0.25 ng/day EE_2 + 2.5 ng/day N, or (4) sesame oil. Adult treatments: At 3 months of age, some C3H animals who received 0.03 µg/day DES, 15 µg/day HPC or oil prenatally and some who received 0.001 µg/day DES, 15 µg/day HPC or oil neonatally were given (1) 0.1 mg/dose or 1.0 mg/dose DMBA in 2 doses, (2) 54 ng/day EE_2 + 540 ng/day N 3 days/week until sacrifice, or (3) the appropriate vehicle. Lactating treatment: Some C3H mothers received 23 ng/day EE_2; 230 ng/day N, 23 ng/day EE_2 + 230 ng/day N, or oil on the first 5 days after birth of young. Animals from each treatment group are being sacrificed monthly for 12 months for C3H animals and 18 months for C3Hf animals. Mammary gland whole mounts are being examined histologically.

Project Officer: Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $95,000 Terminal

CONTRACT RESEARCH SUMMARY

Title: Effect of Mammary Carcinoma Cells on the Vascular Endothelium

Principal Investigator: Dr. Richard R. Lindquist
Performing Organization: University of Connecticut
City and State: Farmington, CT

Contract Number: N01-CB-74089
Starting Date: 8/1/77 Expiration Date: 7/31/80

Goal: To elucidate the cancer cell-endothelium interaction and the effect cancer cells have on the vascular endothelium.

Approach: Human mammary cancer cells in co-culture with endothelial cells will be the model in studies to determine: (a) cancer cell-endothelial interactions using time-lapse phase contrast microscopy and electron microscopy; (b) interacting forces involved; (c) influence of various blood elements and cellular organelles on strength of the interactions; (d) cancer cell-inflicted damage to endothelium; and (e) cancer cell-induced directional proliferation of endothelial cells. Human cells will be obtained from the Cell Bank (BCTF) and endothelial cells will be isolated from umbilical cords.

Progress: The interaction between mammary cancer cells and vascular endothelium was observed in vitro with the aid of time-lapse microcinematography. Cancer cells were added to endothelial cell monolayers and micrographs were taken at either 4- or 8-second intervals. Tumor cells, particularly those at the periphery of tumor cell clumps, have active cell surfaces. Frequent slender cytoplasmic projections constantly form and disappear. These projections, no doubt, correspond to the projections that we have seen by scanning microscopy. Initial contact by the cancer cells with the endothelium occurs via these projections. On establishing contact with the endothelium, individual cancer cells often leave the tumor clump and migrate over the endothelium. Other tumor cells appear to penetrate between endothelial cells and attach to the glass substrate. The cancer cells do this without any evidence of injury to endothelial cells. The endothelial cells, however, actively divide and this division occurs in confluent endothelial monolayers which were contact inhibited before adding the cancer cells. In the coming period we will concentrate on this possible replication of endothelial cells. It could be a response to tumor angiogenic factor.

Project Officer: Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $70,800

CONTRACT RESEARCH SUMMARY

Title: Osteotropism of Mammary Carcinoma Metastasis

Principal Investigator: Dr. Gregory R. Mundy
Performing Organization: University of Connecticut
City and State: Farmington, CT

Contract Number: N01-CB-63980
Starting Date: 6/21/76 Expiration Date: 6/20/79

Goal: To identify the mechanisms by which bone is altered in breast cancer.

Approach: (1) Determine the cellular mechanism of bone destruction in metastatic breast cancer. (2) Determine the hormonal factors responsible for hypercalcemia and/or bone metastases in breast cancer. (3) Characterize the effects of these factors on bone and identify ways in which those effects can be prevented or opposed. In vitro and in vivo systems of human and animal models are utilized.

Progress: (1) The human breast cancer cell line MCF-7 has been found to resorb bone in vitro independent of osteoclast-mediated mechanisms. This has been demonstrated by culture of MCF-7 cells on devitalized fetal rat long bones and by application of MCF-7 supernates to live bone co-cultured with cortisol (10^{-6}M) and phosphate (6 mM), both of which inhibit osteoclast activity. The molecular mechanisms by which these breast cancer cells resorb bones is now under study.

(2) Monocytes and macrophages are often found adjacent to breast cancer metastases. Recently we found that these cells also directly resorb devitalized bone in vitro (Science 196:1109, 1977), which may explain some of the bone resorption which occurs in metastatic breast cancer. The molecular mechanism of monocyte-mediated bone resorption is under study. The mechanism by which circulating monocytes are attracted to sites of bone remodeling have been examined using Boyden chambers and the technique of chemotaxis under agarose. We have found that media which have been cultured with resorbing fetal rat long bones contain factors which are chemotactic to monocytes, but not to neutrophils or lymphocytes. The mechanism by which monocytes are attracted to bone surfaces may be of fundamental importance to the process of bone resorption.

(3) Bone is one of the most common sites for the metastasis of tumor cells, and particularly breast cancer cells. To examine the pathogenesis of bone metastasis, we have developed an in vitro model using cultured rat breast tumor cells that frequently metastasize to bone and cause hypercalcemia (Walker 256 cells). We have found that bone contains a factor which has potent chemotactic activity for these tumor cells. These results may relate to the propensity of malignant cells to metastasize selectively to bone.

Project Officer: Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: 0 Terminal

CONTRACT RESEARCH SUMMARY

Title: Structure Function Relationship of Prolactin Interactions in Mammary Gland Cells

Principal Investigator: Dr. Kurt E. Ebner
Performing Organization: University of Kansas
City and State: Kansas City, KS

Contract Number: NO1-CB-63985
Starting Date: 6/14/76 Expiration Date: 10/13/80

Goal: To modify ovine prolactin and determine the critical amino acids involved in binding of the hormone to membranes, and stimulation of α-lactalbumin formation.

Approach: Modify prolactin chemically by using mercaptoethanol, N-ethylmaleimide, iodoacetamide, cyanogen bromide, tetranitromethane, carbodiimide. Use trypsin, chymotrypsin, plasmin, pepsin, and leucine amino acid peptidase for enzyme modification of prolactin. Modifications will include tyrosine, tryptophan, lysine, methionine, histidine, carboxyl groups, and reduction of disulfides. Prolactin activity will be assayed by binding to mammary particles and to stimulate α-lactalbumin synthesis in mid-pregnant mouse mammary explants.

Progress: A major effort was concerned with refining the biological assay for prolactin, which is the stimulation of α-lactalbumin in mouse mammary mid-pregnant explants. Efforts were also made to develop an assay for prolactin by utilizing mammary cells from mid-pregnant mice. These cells were supported on a collagen matrix and preliminary experiments showed they produce α-lactalbumin in response to prolactin.

A possible assay for the prolactin receptor was examined by making use of fluorescence polarization. That is, fluorescein-prolactin was used as the fluorescence probe. An apparent interaction occurred as monitored by an increase in polarization. Subsequent work showed that this binding could not be displaced by excess prolactin and that the fluorescein-prolactin bound to bovine serum albumin which interfered with the development of an assay.

Further studies were conducted on attempts to define a binding domain in prolactin. Modification of the tyrosine residues resulted in no loss of activity unless nitration was done under denaturing conditions. There was no loss of activity in prolactin when five histidine residues were modified; however, modification of the last two histidines result in complete loss of activity. Further experiments suggest that the site responsible for the lactogenic effects are located in the N-terminal region of both prolactin and those growth hormones that are lactogenic.

Project Officer: Chester V. Piczak, B.S., Barbara Vonderhaar, Ph.D.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: 0 Terminal

CONTRACT RESEARCH SUMMARY

Title: Purification of Estrogen Receptor in Tangible Amounts

Principal Investigator: Dr. Giovanni A. Puca
Performing Organization: University of Naples
City and State: Naples, Italy

Contract Number: NO1-CB-64074
Starting Date: 9/30/76 Expiration Date: 5/29/80

Goal: To isolate and purify the "native" form of estrogen receptor in milligram quantities.

Approach: Carry out large-scale tissue disintegration and centrifugation for isolation of estrogen receptor from calves' uteri. Purify the "native" form of estradiol receptor by a combination of affinity chromatography on new adsorbents and conventional separation methods.

Progress: A method has been worked out for the purification to homogeneity of estrogen receptor from calf uterus cytosol. This method consists of affinity chromatography on heparin-agarose followed by affinity chromatography on an estrogen-containing adsorbent, by a second adsorption on heparin-agarose, and finally by gel filtration on Sephadex G-50. Elution from heparin-agarose is obtained using buffer containing heparin (4 mg/ml), and from the estrogen-adsorbent using buffer containing 0.5 M NaSCN and estradiol. The yield from 1 kg of uteri is from 0.5 to 1 mg of receptor protein, depending on uteri, with a recovery that can be as high as 50%. A single protein band, co-migrating with the ^{3}H-estradiol, is seen on polyacrylamide gel electrophoresis. A single protein band, with an apparent molecular weight of 70,000, is also seen on electrophoresis in sodium dodecyl sulfate gels. As computed from specific activity, there is one estradiol binding site per 70,000 receptor subunit. The pure receptor on low salt sucrose gradient sediments at 8 S and, on high salt or in chaotropic salt containing gradient sediments, at about 4 S. The high tendency to form aggregates is still maintained by the pure receptor as judged by the elution pattern from Sephadex G-200 columns. Aggregation is avoided by small concentrations of heparin in the buffers.

We are now trying very hard to obtain uteri with high estradiol receptor content to carry out large-scale experiments.

Project Officers: Chester V. Piczak, B.S., D. Jane Taylor, Ph.D.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: 0 Terminal

CONTRACT RESEARCH SUMMARY

Title: Methylation of Transfer RNA in Normal and Neoplastic Mammary Tissue

Principal Investigator: Dr. Phoebe S. Leboy
Performing Organization: University of Pennsylvania
City and State: Philadelphia, PA

Contract Number: NO1-CB-74108
Starting Date: 8/15/77 Expiration Date: 8/14/80

Goal: To examine tRNA methylation in normal mammary gland and DMBA-induced hormone-responsive and nonresponsive tumors in the rat.

Approach: Compare the level of methyltransferase activity in normal quiescent, late pregnant, and lactating mammary tissue and DMBA-induced mammary tumors. Compare patterns of tRNA methyltransferase activities in hormone responsive and nonresponsive tumors with those from non-neoplastic mammary tissue. Purify individual tRNA methyltransferases from mammary tumors and corresponding enzymes from normal mammary gland, and compare their biochemical properties. Test methyltransferase inhibitors in vitro and in vivo to determine the effect on cell or tumor growth.

Progress: The total tRNA methyltransferase activity/mg tissue protein in tumor extracts is three times higher than in normal mammary extracts and about 40% higher than in extracts from late-pregnant mammary gland; however, the relative distribution of individual methyltransferases is the same in all three extracts. The extent of methylation of a limiting amount of tRNA substrate with normal mammary extracts is only 40-70% of that achieved with tumor enzymes. When analyzed with purified tRNAs, this difference appears attributable to an increased capacity of tumor extracts to approach the theoretical maximum of 1 methyl group/site/tRNA molecule in the formation of 5-methyl cytosine and 2-methylguanine.

The 2-methylguanine methyltransferases from mammary tumors, normal mammary glands, and glands from pregnant rats have been purified and their properties compared. The purified tumor enzyme responsible for 2-methylguanine formation at position 10 on tRNAs shows higher extent of methylation than its nonmalignant counterparts and is of larger molecular weight than the comparable enzyme from pregnant mammary gland, benign mammary tumor, or rat liver.

Project Officer: Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $99,800 Terminal

CONTRACT RESEARCH SUMMARY

Title: Biochemical Mechanism of Endocrine-induced Breast Regression

Principal Investigator: Dr. William L. McGuire
Performing Organization: University of Texas
City and State: San Antonio, TX

Contract Number: NO1-CB-23862
Starting Date: 6/1/72 Expiration Date: 5/31/80

Goal: To obtain new information of the hormonal control of breast tumor growth and to identify those breast cancer patients whose disease will respond to hormonal manipulation.

Approach: Investigate the sequence of events from initial estrogen binding to receptor sites to nuclear localization of receptor complex and estrogen stimulation of progesterone receptor complex and estrogen stimulation of progesterone receptor synthesis.

Progress: We have shown in a pilot study that ER provides an independent assessment for the risk of early recurrence and poor survival following mastectomy. This could lead to a new approach to the design and strategy of adjuvant trials in breast cancer. Based on the success of our pilot study we are expanding our series and sending tumor sections to Dr. E. Fisher for a blinded analysis of 36 histopathologic variables. We are also participating in a collaborative clinical study where positive node patients are treated with combination chemotherapy with and without antiestrogen and with and without BCG. All patients have ER determinations in the primary tumor specimen. Early results suggest that antiestrogen plus chemotherapy are superior to chemotherapy alone in ER+ patients.

Using human breast cancer cells in culture, our data demonstrate the retention of functional estrogen receptor as well as biological responses to estrogen and antiestrogen following irradiation. This study suggests that previously irradiated tissues can be used for receptor assays and also that breast cancer patients may still be amenable to endocrine therapy of recurrent and/or metastatic lesions following extensive local radiation therapy.

We are in the process of simultaneously measuring ER and "type II binders" by the standard biochemical techniques and by immunocytochemical methods. To prepare a reagent allowing an unequivocal ICC localization of ER and to permit use of an RIA for ER, we are also proceeding to develop antibodies against ER using the new myeloma hybridoma technique. We have produced our first batch of immune hybridomas and are now screening them for specific antibody activity.

Project Officers: Chester V. Piczak, B.S., E. Brad Thompson, M.D.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $120,000

CONTRACT RESEARCH SUMMARY

Title: Differentiation of Mammary Epithelial Cells

Principal Investigator: Dr. Howard L. Hosick
Performing Organization: Washington State University
City and State: Pullman, WA

Contract Number: NO1-CB-63986
Starting Date: 6/30/76 Expiration Date: 6/29/80

Goal: To study how several mesenchymal cell types in the adult mouse mammary gland interact with mammary epithelium to regulate its development.

Approach: Isolate mammary epithelial cells from C3H mice in midpregnant state. Grow these cells as monolayers in culture dishes and on various permeable substrata. Test the responses of the epithelium, under various endocrine conditions, to other mammary cell types and their products. The emphasis is on interactions with adipose cells and fibroblasts. Responses of epithelium to be measured in terms of growth and differentiation (production of mammary-specific products). Responses of normal, preneoplastic, and neoplastic cells will be compared. Undertake studies for defining conditions for growth and passage of primary cultures of normal and premalignant epithelial cells.

Progress: A. Much of our effort has been focused on inserting unesterified fatty acids, of the kinds which might be liberated from mammary adipose tissue, into mammary epithelial cells in culture. In one approach, liposomes have been constructed containing oleic, linoleic, or arachidonic acid. When these liposomes are added to culture medium, they fuse with apical cell surfaces, fatty acids enter the cells, and they are metabolized. Oleic acid so supplied enhances replication of midpregnant mammary cells by approximately 50%. Arachidonic acid is also marginally stimulatory and linoleic acid has no detectable effect on growth. Also, cells have been grown on permeable substrata through which unesterified fatty acids can percolate to the basal surface of cells to be taken up. Four such substrata have been tested: hollow fiber membranes, diethylaminoethyl chloride-coated Sephadex beads, hydrated collagen gels, and thin layers of collagen supported on nylon mesh. The latter substratum seems most promising as a manipulable support for cell growth. B. Our chromatographic procedure for isolating small quantities of newly-made lactose from culture fluids has been improved. The assay has been used to measure lactose production by as few as 10^6 lactating cells. The assay is to be employed as a marker of differentiation. C. Optimal culture conditions for mouse and human mammary cells are being devised. Embryonic extract improves the quality (epithelial morphology, replication rate) of mouse cells, but as yet has not been effective on human cells (isolated from milk). We have recently found also that the precise amino acid and carbohydrate compositions of culture fluids are very important for maintenance of these cells, so they are being optimized.

Project Officer: Chester V. Piczak, B.S., Barbara Vonderhaar, Ph.D.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $47,600 Terminal

CONTRACT RESEARCH SUMMARY

Title: Glycoproteins of Mammary Cell Surface

Principal Investigator: Dr. Clayton A. Buck
Performing Organization: Wistar Institute
City and State: Philadelphia, PA

Contract Number: NO1-CB-64069
Starting Date: 6/28/76 Expiration Date: 6/27/79

Goal: To determine the synthesis and fate of mammary tumor glycoproteins.

Approach: Glycoproteins will be derived from mouse mammary tumor systems and normal mammary tissue labeled in situ or following growth in culture with ^{3}H- or ^{14}C-labeled sugars. Membrane bound and shed glycoproteins will be analyzed by immunoprecipitation and polyacrylamide gel electrophoresis. Some of these antigens are involved in cell-substratum adhesion. These glycoproteins will be identified on the basis of their ability to block antibody-induced rounding and detachment of cells from the substratum. They will be purified by ion exchange and lectin chromatography for use in the production of monoclonal, monospecific antibodies.

Progress: (1) Immunological Studies: Antisera prepared in goats against shed material from two mouse tumor lines (one virus producing), from one control mouse cell line, and from primary mouse mammary cells have been used to immunoprecipitate material from detergent extracts of both control and mouse mammary cells. These immunoprecipitates were analyzed by polyacrylamide gel electrophoresis and revealed selective precipitation of a group of fucose-labeled glycoproteins with a molecular weight of approximately 120,000 daltons.

Sequential immunoprecipitation experiments show (a) all antisera react with a glycoprotein or glycoproteins of approximately 120,000 MW from membranes of control and tumorigenic cells; (b) antisera against material shed from the virus-producing cell line react with a unique glycoprotein in extracts from tumorigenic cells. The status of the antigen on control cells has not yet been established.

(2) Effects of the Antisera on Cells in Tissue Culture: The antisera produced against shed material from mouse and human mammary tumor when added to growing monolayers change the morphological and adhesive properties of the cells. These effects are entirely reversible and can be blocked by glycoproteins extracted from membrane preparations of the affected cells. Experiments to purify and isolate the blocking antigens from both human and mouse cells are in progress.

Project Officer: Chester V. Piczak, B.S., D. Jane Taylor, Ph.D.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: 0 Terminal

CONTRACT RESEARCH SUMMARY

Title: Isolation and Characterization of Mammary Epithelial Cell Plasma Membrane

Principal Investigator: Dr. Grant Fairbanks
Performing Organization: Worcester Foundation for Experimental Biology
City and State: Shrewsbury, MA

Contract Number: NO1-CB-33908
Starting Date: 6/1/73 Expiration Date: 2/28/79

Goal: Development of new methods for plasma membrane isolation and characterization applicable to normal and malignant mammary cells.

Approach: Membrane modification reagents and activated beads will be used to isolate plasma membrane fragments from ascites cells of the metastatic 13762 rat mammary adenocarcinoma. Macromolecular organization at the extracellular and cytoplasmic faces of the membranes will be analyzed by combined application of selective radiolabeling, protein cross-linking, and enzymatic dissection.

Progress: In the first radiosynthesis of a cleavable protein cross-linking reagent, ^{35}S-dithiobis(succinimidyl propionate) (^{35}S-DTSP) was prepared at 20 mCi/mmole. DTSP combines high reactivity against amino groups with long lifetime in aqueous solution. Under mild conditions (e.g., 1-5 minutes at 0°C, pH 7, with 10^{-5}-10^{-3} M DTSP) it is an efficient probe of protein-protein interaction in membranes and other extended structures (Lomant, A. J., and Fairbanks, G.: J. Mol. Biol. 104:243, 1976). We have combined surface radioiodination (Hubbard, A. L., and Cohn, Z. A.: J. Cell Biol. 64:438 1975), DTSP cross-linking, and two-dimensional electrophoresis (Liu, S.-C., et al.: Biochem. 16:4066, 1977) in studying the surface organization of 13762 cells. Two-dimensional electrophoresis yields "fingerprints" in which three classes of surface components are resolved: (i) proteins with little or no covalently bound sugar, (ii) proteins that are extensively glycosylated, and (iii) very large, highly heterogeneous (10^5-10^6 daltons) glycosaminoglycan species. Most of the surface glycosaminoglycan on cultured 13762 cells is in the form of disulfide-bonded aggregates with apparent MW >10^6. Intrinsic intramolecular cross-linking of two discrete nonglycosylated proteins has also been noted. Treatment with exogenous cross-linker, DTSP, results in extensive cross-linking of internal proteins, but little cross-linking of ^{125}I-labeled surface proteins. We conclude that, although these accessible surface components are tightly attached, they present themselves as free-floating monomers and do not form multimeric structures involving extensive, specific protein-protein interaction.

Project Officers: Chester V. Piczak, B.S., Barbara Vonderhaar, Ph.D.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: 0 Terminal

CONTRACT RESEARCH SUMMARY

Title: Produce and Identify Antibodies to Collagens/Procollagens and/or Related Enzymes

Principal Investigator: Dr. Heinz Furthmayr
Performing Organization: Yale University
City and State: New Haven, CT

Contract Number: N01-CB-84225
Starting Date: 9/18/78 Expiration Date: 9/17/80

Goal: To prepare immunochemically defined antibody reagents to collagen of types I, II, III, IV (basement membrane), AB (fetal collagen), procollagens of types I and III, and mouse collagen of types I, III, and IV.

Approach: Studies include: (1) isolation and purification of collagens from calf, human, and mouse tissue; (2) immunization of rabbits and sheep; (3) serological screening of antisera during immunization and after final bleeding; (4) isolation and purification of antibodies specific for each collagen; (5) characterization of the isolated antibodies; and (6) storage and distribution of the purified antibodies to other investigators.

Progress: Various amounts of different types of collagen from calf skin and nasal cartilage, human placenta, and a transplantable tumor in mice were isolated. These include sufficient amounts of type I and type II collagen from calf; 2 g of type I, 2 g of type III and 200 mg of type V (AB_2) collagen from human amnionic and chorionic membranes. Obtained 500 g of the EHS sarcoma grown in mice and stored frozen at -70°C for isolation of mouse type IV collagen. Skins from 200 mice treated with β-aminoproprionitrile were prepared and frozen at -70°C for future isolation of mouse type I and type III collagen. A NaCl extract from skins of dermatosparactic cattle obtained from Dr. Charles Lapiere will serve as starting material for isolation of pα1 chains. Established methods for isolation and purification of all of the collagens mentioned above. Four groups of 10 rabbits were immunized with calf type I, calf type II, human type III, and human type V collagen, and each antiserum was tested by hemagglutination throughout the course of immunization. Serum was collected and stored at -30°C. Two sheep were hyperimmunized with rabbit IgG for preparation of a potent anti-rabbit IgG serum. Approximately 3500 ml of serum was obtained and stored frozen at -30°C. Cells from muscle fibroblast and endothelium were isolated from calf aorta and grown in tissue culture. Each cell type produces more than one type of collagen as shown by labeling of the cells in indirect immunofluorescence experiments. Incorporation of ^{3}H- and ^{14}C-proline and/or glycine into newly synthesized collagen in combination with antibody precipitation techniques and analytical SDS-polyacrylamide electrophoresis was used to determine specificity of highly purified collagen antibodies.

Project Officer: Chester V. Piczak, B.S.
Program: Breast Cancer Experimental Biology
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $82,200

CONTRACT RESEARCH SUMMARY

Title: Cell Kinetics of Breast Cancer

Principal Investigator:	Dr. Lewis M. Schiffer
Performing Organization:	Allegheny General Hospital
City and State:	Pittsburgh, PA
Contract Number:	NO1-CB-43899
Starting Date:	2/1/74
Expiration Date:	4/14/79

Goal: To develop and exploit methods of rapidly determining cell kinetic parameters of individual human breast cancers so that this information may potentially be used in the study of the natural history and treatment of these tumors.

Approach: Three cell kinetic parameters of human and non-human solid breast tumors are measured directly by tumor imprints and mechanical disaggregation of tissue followed by *in vitro* incubation with isotopically labeled thymidine. These parameters are ^{3}H-TdR labeling index, DNA synthesis time, and PDP index (which estimates growth fraction). Potential doubling time and cell cycle time can be calculated from these values. Other techniques, such as autoradiographic grain counting and freezing to transport specimens, are being developed to pursue the goal of clinical utility. A data base of human breast tissue, both benign and malignant, has been developed and correlated with clinical parameters.

Progress: The results of this project can be summarized in six categories:

1. Techniques were developed to obtain cell kinetic values from biopsies of animal and human tumors in clinically useful time frames. These methods were successful and are in continuous use.
2. The cell kinetics of an unperturbed spontaneous mammary tumor system were defined for the first time, using hundreds of C3H/He tumors from which individual results were obtained.
3. The cell kinetic responses of the C3H/He system to perturbation by single chemotherapeutic drugs, radiation, surgery, steroids, and nutritional changes were defined. The use of multiple agents on a time-sequenced basis is proving more successful than agents given simultaneously.
4. The cell kinetics of the 13762 transplantable rat mammary tumor were defined, along with similar cell kinetic perturbation data for chemotherapeutic drugs.
5. The cell kinetics of individual unperturbed human breast tumors were studied, and correlations made with clinical parameters.
6. Studies of animal and human metastases, simplified techniques for cell kinetic data acquisition, and transportability of human specimens are in progress.

Project Officers: Mary E. Sears, M.D., Stanley Shackney, M.D.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Methods To Predict Chemotherapy Sensitivity

Principal Investigator: Dr. Lewis M. Schiffer
Performing Organization: Allegheny General Hospital
City and State: Pittsburgh, PA

Contract Number: NO1-CB-74082
Starting Date: 7/1/77 Expiration Date: 6/30/80

Goal: To develop a reliable test system for predicting the effectiveness of chemotherapeutic agents in the treatment of individual breast cancers.

Approach: The experiments on two animal tumor models, the DMBA-induced rat mammary tumor and the C3H/He mouse spontaneous mammary tumor, will test the correlation of unperturbed tumor cell kinetics and tumor response in individual animals to chemotherapeutic agents. The hypothesis to be tested is that the highly variable cell kinetic parameters are related to the drug responsiveness of tumors. The cell kinetic studies will include tritiated thymidine labeling index, DNA synthesis time, and tumor growth fraction estimation via primer-available DNA polymerase assay.

Progress: More than 100 DMBA tumors have been analyzed for the basic technical studies. Cell kinetic comparison of excisional and needle biopsy shows no significant differences. Alterations in cell kinetics become normalized at 72 hours. The mean tumor doubling time was 40.7, and median 19.9 days with a log-normal distribution. Well-differentiated tumors had lower ^{3}H-TdR and PDP indices than did poorly differentiated tumors.

Thirty to 38 animals with single or multiple tumors were registered in each therapeutic group. Injections were made i.p. with the exception of adriamycin. We found adriamycin i.p. injection to be toxic and gave it i.v. to the full complement of animals. Animal tumors were measured with calipers 3 times weekly until they regained double the tumor value at the time of treatment. The doses used were: 5-FU 100 mg/kg, Cytoxan 80 mg/kg, VCR 0.5 mg/kg, MTX 60 mg/kg, adriamycin 5 mg/kg, and methylprednisilone 10 mg/kg ql2h x 3. All studies are complete except for methylprednisilone, which is ongoing. There is a great variation among the individual cell kinetic patterns, tumor volume reductions as a result of chemotherapy, and tumor regrowth patterns.

Project Officers: Mary E. Sears, M.D., Stanley Shackney, M.D.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $115,000

CONTRACT RESEARCH SUMMARY

Title: Therapy for Stage II or Stage III Carcinoma of the Breast

Principal Investigator: Dr. Charles A. Hubay
Performing Organization: Case Western Reserve University
City and State: Cleveland, OH

Contract Number: NO1-CB-43990
Starting Date: 6/16/74 Expiration Date: 6/15/80

Goal: To test the effects of adding chemotherapy, antiestrogen therapy, and BCG to mastectomy in patients with locally advanced breast cancer (Stages II-III).

Approach: Patients under age 76 who show axillary nodes involved with metastases at the time of surgery are eligible for this study. Stratification, but not treatment selection, is on the basis of the presence or absence of estrogen receptor protein (ER) in the tumor. Random treatment assignments are (1) cyclophosphamide, methotrexate, 5-fluorouracil (CMF); (2) cyclophosphamide, methotrexate, 5-fluorouracil, tamoxifen (CMFT) for 12 months; and (3) CMFT for 12 months plus BCG for 12 months. Endpoint is the first evidence of treatment failure, i.e., the appearance of locally recurrent or of distant tumor.

Progress: To date, 284 patients have been enrolled in the study. Of these, 76% had estrogen receptor (ER)-positive tumors (>3 femtomoles/mg of protein). At the present time, 136 patients have completed one year of therapy. Of the treated patients, 15 have died and 43 have had treatment failure. Thirty patients have withdrawn voluntarily after one month to one year of therapy. Chemotherapy has not been accompanied by significant myelosuppression. Hair loss has been slight. Tamoxifen, the antiestrogen agent used, has been tolerated well, without serious side effects, although most patients experience hot flashes while on the drug. Forty-three patients have had recurrence: 20 in the CMF group, 11 in the CMF + tamoxifen, and 12 in the CMF + tamoxifen + BCG group. Statistical comparison for treatment failure between Stage II ER-positive and ER-negative patients is significant at the $p<.0001$ level, with the latter group recurring more rapidly (38.2% vs. 22% at 30 months). Relapse rate for the Stage II treated patients at 30 months is as follows: CMF - 38.1%, CMF + tamoxifen - 15.5%, and CMF + tamoxifen + BCG - 24%. Recurrence rates between Stage II patients with 1-3 positive nodes are significantly different when stratified according to estrogen receptor values, with higher relapse rates in the ER-negative group. There is no significant difference between the pre- and postmenopausal groups. A number of patients have been followed for greater than 24 months, allowing statistical comparison of all patients in positive and negative groups with 1-3 positive nodes and ≥ 4 positive nodes.

Project Officers: Mary E. Sears, M.D., Ernest deMoss, M.D.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $110,000

CONTRACT RESEARCH SUMMARY

Title: Therapy of Patients with Stage II or Stage III Carcinoma of the Breast

Principal Investigator:	Dr. Edward F. Scanlon
Performing Organization:	Evanston Hospital
City and State:	Evanston, IL
Contract Number:	NO1-CB-53917
Starting Date:	6/30/75
Expiration Date:	6/29/80

Goal: To determine the effects of adding chemotherapy and BCG to mastectomy for patients with locally advanced breast cancer.

Approach: Patients who have metastatic axillary nodes or Stage III breast cancers are admitted to the study program. Stratification and randomization are carried out along with a balancing for prognostic factors according to the following variables: Primary tumor size, number of positive nodes, menstrual status, and unfavorable local signs. The statistical analysis involves sequential treatment assignment and is designed to provide the greatest balance between all groups assigned according to all available prognostic indicators. One of three treatment schedules is assigned to each of the patients in the study according to the above computer classifications: (1) Intermittent phenylalanine mustard (PAM); (2) 5-fluorouracil, cyclophosphamide, and prednisone in intermittent courses; and (3) the three-drug chemotherapy plus BCG inoculations. Two of the three treatment schedules are identical with the Mayo Clinic adjuvant study and the data will be cumulative. The statistical method of analysis is also identical with that now being used at the Mayo Clinic. The treatment courses will be given for one year or until there is evidence of relapse, whichever occurs first. Immunologic evaluation is done initially and every three months. Survival and recurrence-free intervals will be computed for each treatment group.

Progress: Since July 1975, the study has enrolled 163 patients. Entry to the L-PAM regimen was discontinued in November 1977 because of a disproportionately high tumor recurrence rate, 12 of 38 (32%) compared to 6 of 60 (10%) in Group II and 6 of 60 in Group III (10%). Of those patients with recurrences, 17 of the 24 patients had primary tumors larger than 3 cm, and 15 had four or more metastatic axillary nodes. The patients who received polychemotherapy had a higher frequency of nausea, vomiting, diarrhea, alopecia, and stomatitis than those who received L-PAM; however, L-PAM results in more severe thrombocytopenia. Immunologic evaluation was done initially and every three months for the first two and a half years of the study. However, phytohemagglutination analysis and skin tests do not reveal any significant trends among treatment groups.

Project Officers: Mary E. Sears, M.D., Donald Henson, M.D.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $79,200

CONTRACT RESEARCH SUMMARY

Title: Steroid Sulfation and Estrogen Binding in Human Breast Cancer

Principal Investigator: Dr. Thomas L. Dao
Performing Organization: Health Research, Inc.
City and State: Buffalo, NY

Contract Number: NO1-CB-43900
Starting Date: 2/1/74 Expiration Date: 1/31/80

Goal: To determine the relationship of steroid sulfotransferase activity to estrogen receptor protein levels, to pathologic staging, and to risk of relapse.

Approach: Steroid sulfating enzyme activity and estrogen receptor (ER) levels have been assayed on breast tissue obtained from approximately 300 patients in three institutions. The evidence will be evaluated for or against correlation of the steroid sulfotransferase activity with risk for tumor metastasizing potential and with histopathological parameters. The predictive potentials of ER and sulfation activity will be compared.

Progress: A total of 266 patients from three cooperating institutions have been entered into the study between March 1974 and June 1976, inclusive. The average length of follow-up is now approximately 32 months. A total of 25 patients were excluded for the following reasons: (1) the patients received treatment not described in the protocol; (2) they did not receive primary treatment as described in the protocol; (3) they were lost to follow-up. Based on an analysis of 205 patients from two institutions (the follow-up of 37 patients from the third institution is not complete and, therefore, is not included in this report), the data disclose that (1) there was a 36% recurrence rate in patients with ER-negative tumors as compared with a recurrence rate of 37% in patients with ER-positive tumors; (2) in premenopausal women, the recurrence rate is 17% in ER-negative patients and 44% in patients with ER-positive tumors. In contrast, the recurrence rate in postmenopausal women is much higher in those with ER-negative tumors than in those patients with ER-positive tumors (43% vs. 36%). (3) The data suggest that the recurrence rate is related to a low level of DHEAS, particularly in patients with no lymph node metastasis; thus, recurrence has occurred in 23% of the patients whose DHEAS levels are less than 25 pg/mg protein and in 13% of the patients whose tumors synthesize much higher levels of DHEAS (>25 pg/mg protein). Longer follow-up is needed to confirm or refute the prognostic significance of steroid sulfating and ER activity in the tumor.

Project Officers: Mary E. Sears, M.D., D. Jane Taylor, Ph.D.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $10,000

CONTRACT RESEARCH SUMMARY

Title: Predictive Transplantable Animal Mammary Tumor Models

Principal Investigator: Dr. Arthur E. Bogden
Performing Organization: Mason Research Institute
City and State: Worcester, MA

Contract Number: NO1-CB-43914
Starting Date: 11/1/73 Expiration Date: 10/31/78

Goal: To carry out preclinical evaluations of single and combined therapeutic modalities in experimental mammary tumors of known biological characteristics.

Approach: Studies are being done in six transplantable rat mammary tumor systems having a spectrum of histological, growth, and metastasizing characteristics. The highly metastatic 13762 mammary adenocarcinoma is used most extensively, and the R3230AC, SMT-2A, 3M2N, DMBA#1, and DMBA#14 mammary tumors are used to test further and evaluate those drug and therapy combinations showing promising activity in the 13762 system. Chemotherapy assays versus subcutaneous grafts are made by initiating drug treatment on the day following implantation or on day 15 when tumors are well established. Metastasis assays combine surgical extirpation of tumors with the modalities of chemotherapy, X-irradiation and immunotherapy, alone and in various combinations. The parameters of immunosuppression and hormone radioimmunoassay are applied in studies requiring in-depth drug and therapy evaluation. Chemotherapeutic agents of current interest include phenylalanine mustard (PAM), adriamycin (ADR), Cytoxan (CTX), 5-fluorouracil (5-FU), methotrexate (MTX), dibromodulcitol (DBD), hexa-methylmelamine (HMM), vincristine (VIN), and the nitrosoureas.

Progress: The growth of the 13762E tumor subline (DES responsive) was stimulated when implanted into ovariectomized (ovexed) hosts 2 to 3 days post ablation. Growth stimulation coincided with a surge in serum total estrogen levels 5 days post ovex which peaked in 10 to 18 days, dropping to subnormal levels by days 30 to 35, suggesting the advisability of a short period of antiestrogen therapy following surgical ablation in the treatment of E_2 receptor-positive patients. However, rate of uterine atrophy was highest during period of peak estrogen (?) surge. An acute treatment regimen with L-PAM or VIN increased estrogen levels in the ovexed animal. A treatment sequence in which a short course of chemotherapy (L-PAM) preceded surgery that was followed by 3Rx with levamisole produced 91% cures and significant prolongation of lifespan in the 13762 metastatic tumor system. Preliminary studies indicate that primary surgical explants of human breast cancers show positive growth as xenografts in the 6-day subrenal capsule assay using normal immunocompetent mice. Data supporting the feasibility of a predictive chemotherapy assay are being accumulated.

Project Officers: D. Jane Taylor, Ph.D., Chester V. Piczak, B.S.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Data Research Analysis for Breast Cancer Treatment Program

Principal Investigator: Ms. Marlene Dunsmore
Performing Organization: Mason Research Institute
City and State: Rockville, MD

Contract Number: NO1-CB-74140
Starting Date: 3/21/77 Expiration Date: 3/20/80

Goal: To increase the usefulness of data produced in projects related to the treatment of human breast cancer.

Approach: A central data file will be set up for three areas of study: (1) surgical adjuvant therapy, (2) tumor markers, and (3) estrogen receptor assays. The file will allow comparison of the results from various studies and provide a data base from which material can be quickly and conveniently retrieved when desired for testing new ideas, identifying groups of patients suitable for more detailed study, and for preparing reports to the medical and general public. The file is not intended to duplicate those at individual institutions but to perform analyses that are not possible at the individual institutions.

Progress: Mason personnel have designed a data collection and editing system for the Breast Cancer Studies Data Center. Clinical information is abstracted and entered via an IBM on-line disk file. The main file update system modifies clinical history files with new information and continues to edit for data consistency. Data concerned with treatment, histopathology, survival, and estrogen receptor status have been stored on 2,186 patients. Personnel prepared extensive tabulations of these data for a meeting of the Breast Cancer Task Force Committee and an analysis of the relationship of estrogen receptor with clinical and survival data for two institutions.

In February 1978, work was begun in support of the Biological Markers Project. The basic data collection and editing systems are now operative. Background and clinical data have been gathered on 5,771 patients from three institutions. Mayo Clinic is serving as the repository for the serum samples.

Project Officers: Mary E. Sears, M.D., Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $124,523

CONTRACT RESEARCH SUMMARY

Title: Surgical Adjuvant Chemotherapy in Patients with Breast Cancer

Principal Investigator: Dr. David L. Ahmann
Performing Organization: Mayo Foundation
City and State: Rochester, MN

Contract Number: NO1-CB-33899
Starting Date: 6/30/73 Expiration Date: 6/29/80

Goal: To assess the effects of surgical adjuvant chemotherapy using a multiple drug program: cyclophosphamide, 5-fluorouracil, and prednisone (CFP), versus CFP plus irradiation therapy versus a program using phenylalanine mustard alone.

Approach: Patients with unfavorable primary breast cancers (node +) are assigned to treatment programs involving mastectomy followed by multiple drug chemotherapy (CFP), multiple drug chemotherapy (CFP) with postoperative irradiation, or single agent chemotherapy using phenylalanine mustard. After stratification according to menstrual category, tumor size, and degree of involvement, each patient is randomly assigned to one of the three treatment programs as indicated above. The adjuvant chemotherapy is initiated two to four weeks postoperatively and given concomitantly with radiation therapy when this modality also is employed. Chemotherapy is terminated at the end of one year (10 courses) or upon the appearance of recurrent disease, whichever occurs first.

Progress: A total of 256 patients have entered on study, 250 of which are evaluable. Seventy patients thus far show recurrence. Recurrence rates per 1,000 weeks of observation are 5.0 for phenylalanine mustard, 2.9 for CFP, and 2.2 for CFP with radiation therapy. The death rate for 1,000 weeks of observation is thus far 1.7 for phenylalanine mustard, 3.1 for CFP, and 2.1 for CFP with radiation therapy. In the premenopausal group of patients CFP with or without radiation statistically is superior to phenylalanine mustard and hence this arm had been dropped for the premenopausal group of patients. The difference between recurrence rates and death rates for the entire group is almost totally accounted for with the difference in the premenopausal group of patients. However, longer follow-up will be needed to determine the effects of radiation therapy on disease-free status and on survival. At the present time, radiation therapy added to the polychemotherapy program does not detract from the program and perhaps might enhance the treatment program. Myelosuppression occurs in most patients and equally distributed among the various groups. Thus far, only about 25% of the premenopausal patients have had significant menstrual disturbances, suggesting again that kinetic factors of tumor as well as hormonal changes may exert influences on ultimate disease-free status and survival.

Project Officers: Mary E. Sears, M.D., Ernest deMoss, M.D.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $75,000

CONTRACT RESEARCH SUMMARY

Title: Occult Breast Cancer Metastases in Axillary Lymph Nodes

Principal Investigator: Dr. Edward J. Wilkinson
Performing Organization: Medical College of Wisconsin
City and State: Milwaukee, WI

Contract Number: NO1-CB-74110
Starting Date: 7/1/77 Expiration Date: 6/30/79

Goal: To determine if the presence of occult lymph node metastases in axillary lymph nodes previously diagnosed as negative correlates with clinical recurrence of breast cancer.

Approach: The investigators are comprehensively restudying the lymph nodes removed from approximately 500 patients at the time of mastectomy for primary breast cancer. In all cases the initial routine histological examination of the nodes demonstrated no evidence of metastases. A pilot study on a subset utilized 6 micron serial sectioning of node material and the best spacing between sequential subsets of these slides has been determined by reevaluation of the subsets. Five years or more follow-up information is available on all patients. The study considers the following factors in relation to their risk for clinical recurrence: primary tumor type, grade and size, breast lymphatic or blood vessel invasion, number of nodes removed, histologic character of the nodes, number of positive nodes and levels, volume of tumor in the nodes, pretreatment and post-treatment records, age, menstrual history, reproductive history, height, weight, and survival information.

Progress: Resectioning of lymph nodes as of the above date has been completed on 430 patients. To date, a total of 95,243 slides have been sectioned for this study. All slides are being sectioned at 6 micron thickness. Within the pilot study the first 57 cases were serially sectioned using an average of 403 slides per case. From case 58 and on, sections were spaced at 24 micron spacing. An average of 262 slides have been cut per case with this spacing. Cases 1 through 57 were read by the pathologist as described in the pilot study. Cases 58 through 200 were read by reading every 24 micron section. Analyzing the size and character of the 39 occult tumors identified in the first 200 patients, we have determined that reading at 48 micron spacing provides comparable precision at 24 micron spacing when reading, as we predicted in the pilot study. All cases continue to be cut at 24 micron spacing, and from case 201 forward all cases are read at 48 micron spacing. We have complete data on 310 cases and within these 310 cases we have identified occult tumor in 53 cases.

Project Officers: Mary E. Sears, M.D., Donald Henson, M.D.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Suppression of Endocrine Function by Systemic Agents for Breast Cancer Therapy

Principal Investigator: Dr. Richard J. Santen
Performing Organization: Pennsylvania State University
City and State: Hershey, PA

Contract Number: NO1-CB-53851
Starting Date: 5/15/75 Expiration Date: 5/14/80

Goal: To produce suppression of endocrine function with aminoglutethimide (AG), to establish the mechanism of steroid inhibition, and to compare the effects of surgical adrenalectomy, antiestrogen therapy, and aminoglutethimide in human breast carcinoma.

Approach: Female patients with inoperable, recurrent, or metastatic breast carcinoma and whose tumors are either estrogen receptor positive or unknown are selected for study. Women are randomized into two separate therapeutic trials: a comparison of medical adrenalectomy (AG + hydrocortisone) vs. surgical adrenalectomy, and of AG vs. the antiestrogen, tamoxifen. Extensive endocrine studies evaluate the effects of AG on extra-adrenal estrogen production, androgen, progestin, and prolactin secretion, and steroid metabolism.

Progress: A total of 220 women with breast carcinoma have been entered into study in which 73 were randomized to either surgical adrenalectomy or medical therapy with AG. Although follow-up is limited, similar response rates to the two therapies are being observed. Endocrine kinetic studies revealed an important new site of action of AG to inhibit extraglandular estrogen production in addition to its known blocking effects. Consequently, the levels of estrone and estradiol in plasma and urine were suppressed by AG to the same extent as observed in postmenopausal or castrated women after adrenalectomy. Even after surgical ablative therapy women continued to produce estrogens, although in ng rather than mcg amounts. This finding is of great potential importance regarding antiestrogen therapy. AG was found to enhance the activity of the 3β-ol dehydrogenase-Δ^5-Δ^4 isomerase pathway in the adrenal. As a result, androgen and progestin levels were higher in women treated with AG than with surgical adrenalectomy. This finding in concert with response data from the clinical trial should provide unique information regarding the relative importance of endogenous androgens and progestins in mediating mammary tumor regression. Overall, these studies suggest that medical treatment with aminoglutethimide and hydrocortisone is an appropriate alternative to surgical adrenalectomy.

Project Officers: Mary E. Sears, M.D., E. Brad Thompson, M.D.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $180,000

CONTRACT RESEARCH SUMMARY

Title: Hormone Manipulation Plus Chemotherapy in Breast Cancer Patients

Principal Investigator: Dr. Arthur H. Rossof
Performing Organization: Rush-Presbyterian-St. Luke's Medical Center
City and State: Chicago, IL

Contract Number: NO1-CB-43991
Starting Date: 6/17/74 Expiration Date: 6/16/79

Goal: To evaluate the relative merits of early versus delayed combination chemotherapy in patients with metastatic breast cancer known to have responded to surgical oophorectomy.

Approach: Premenopausal patients with metastatic progressing breast cancer are evaluated 12 weeks following surgical castration. Those patients who are showing no change or tumor regression (partial or complete response) are randomly assigned to the maintenance phase of either (1) observation or (2) cyclic treatment courses of cyclophosphamide, 5-fluorouracil, and methotrexate (CMF).

When relapse is documented in patients assigned to observation, CMF treatment will be started. The results of these studies are expected to compare regression rate, time to progression, and survival in these two groups of patients.

Progress: One hundred twelve patients have been registered for the oophorectomy phase of this study. Forty-one patients have entered the maintenance step and seven have subsequently entered the crossover step. Thirty-eight percent of the patients have achieved complete (5 patients, 5%) or partial (16 patients, 16%) response or stable disease (17 patients, 17%) 12 weeks post oophorectomy.

Only six deaths have occurred among the 31 analyzable randomized cases. Thus, it is still too early to analyze survival or even estimate median survivals. Estimating that 75% of the patients will live at least two years after oophorectomy, there will probably not be enough survival data available to write a final report for this study for another two years.

However, with 18 failures noted among the randomized patients, a preliminary analysis of time to first failure can now be made. After adjusting for certain covariates, a Cox model indicates that coded treatment "Y" had a significantly longer time to failure than did treatment "X" ($P = 0.02$).

There has been one case of life-threatening leukopenia on crossover CMF. There have been no deaths due to toxicity.

Project Officers: Mary E. Sears, M.D., Ernest deMoss, M.D.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Therapy of Patients with Stage II Carcinoma of the Breast

Principal Investigator: Dr. Donald L. Morton
Performing Organization: University of California
City and State: Los Angeles, CA

Contract Number: NO1-CB-43917
Starting Date: 6/17/74 Expiration Date: 6/16/80

Goal: To evaluate the effectiveness of adding chemotherapy or chemoimmunotherapy stimulation to mastectomy for patients with Stage II carcinoma of the breast.

Approach: Patients with any number of positive axillary lymph nodes are eligible to enter this study, which randomly assigns one of the following treatment schedules: (1) cyclophosphamide, methotrexate, and 5-fluorouracil (CMF); (2) CMF plus BCG; or (3) CMF plus BCG plus tumor cell vaccine. The tumor cell vaccine consists of irradiated allogeneic breast carcinoma cells grown in tissue culture. CMF is given in 12 cycles over 64 weeks. Immunotherapy is given for 114 weeks. Treatment failure consists of either metastases or development of a new carcinoma in the contralateral breast. The patients undergo evaluation of immunologic parameters before treatment begins and at intervals throughout the study.

Progress: One hundred thirty-two patients have been entered into the protocol since its inception in June 1974. Patients have been randomized into one of the three areas as follows: CMF, 37 patients; CMF + BCG, 55 patients; and CMF + BCG + TCV, 40 patients. The longest follow-up is 53 months. Thirty-three patients have developed metastases, seven occurring while on treatment. Three other patients have developed a second primary in the contralateral breast.

About 80% of patients have had serial testing of immunocompetence by measuring delayed hypersensitivity to DNCB and PPD. The increased reactivity to repeated challenges to DNCB continues to be less in breast cancer patients receiving CMF and BCG compared to a group of melanoma patients receiving only BCG. A historical control group of 58 patients, treated only with surgery for Stage II breast cancer, has been developed to assist in comparing the results of this study with those of similar adjuvant therapy trials.

Project Officers: Mary E. Sears, M.D., Ernest deMoss, M.D.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $75,000

CONTRACT RESEARCH SUMMARY

Title: Prediction of Hormone Dependency in Human Breast Cancer

Principal Investigator: Dr. Elwood V. Jensen
Performing Organization: University of Chicago
City and State: Chicago, IL

Contract Number: NO1-CB-43969
Starting Date: 6/16/66 Expiration Date: 6/15/80

Goal: To develop techniques which will increase the predictability of response of human breast cancers to endocrine therapy.

Approach: Since 1966, Dr. Jensen has been studying estrogen receptors (ER) in human breast cancer tissues and the relationship between ER content and response to endocrine manipulative treatments. Because it now appears that patients whose breast cancers contain small or negligible amounts of ER have little if any chance of responding to endocrine therapy, whereas most but not all patients with tumors containing substantial amounts of ER receive benefit from hormonal manipulation, attention is directed toward three principal objectives: (1) understanding of why some receptor-containing breast cancers do not respond; (2) development of a simple and specific radioimmunoassay for ER in breast cancers; and (3) evaluation of the ability of an ER assay on the primary tumors, carried out at the time of mastectomy, to predict subsequent response to endocrine therapy.

Progress: Of more than 1,200 patients whose primary and/or metastatic breast cancers have been analyzed, approximately 70% can be classed as ER-poor and 30% as ER-rich. During the past several years, ER assays have been carried out on approximately 700 mastectomy patients who had no indication of distant metastases. These women are being followed for recurrence of disseminated cancer and their response to endocrine therapy at that time. So far 11 of 15 patients with ER-rich primary tumors showed objective remission and one showed subjective remission to endocrine therapy at the time of recurrence (median recurrence 24 months) in contrast to only one remission in 22 patients with ER-poor tumors (median recurrence 12 months). Antibodies to purified ER from calf uterus have been found to react with the estrogen receptor complex of human breast cancer cytosol; development of an ER assay in tumor slices by fluorescent antibody technique and in tumor cytosol by competitive RIA is in progress. Important to this effort is the preparation of highly purified or monospecific antibody to estrophilin, which is being attempted by the Milstein technique of cloning a hybridoma cell line obtained by the fusion of lymphoid tissue from immunized animals (rabbits and rats) with mouse myeloma cells.

Project Officers: Mary E. Sears, M.D., Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $85,000

CONTRACT RESEARCH SUMMARY

Title: Effect of Chemotherapy-induced Endocrine Alterations on Stage II Breast Cancer

Principal Investigator:	Dr. Tapas K. DasGupta
Performing Organization:	University of Illinois
City and State:	Chicago, IL
Contract Number:	NO1-CB-84221
Starting Date:	7/1/78
Expiration Date:	6/30/80

Goal: To determine whether the prolongation of the clinically disease-free interval produced by adjuvant chemotherapy is a result (1) of direct anti-cancer action, (2) of indirect action via endocrine organ changes, or (3) of a combination of 1 and 2.

Approach: (1) In the clinical study, premenopausal women 50 years of age or younger with potentially operable breast cancer will be tentatively included for the purpose of obtaining preoperative hormone profiles. Blood sampling, when possible, will be done in both the follicular phase and the luteal phase. Radical or modified radical mastectomy will be performed. If one or more axillary nodes contain histologically proven metastases, the patient is eligible to enter the adjuvant chemotherapy program. Estrogen and progesterone receptor assays will be performed upon the breast cancer tissue. The study patients will receive 18 courses of cyclophosphamide, methotrexate, and fluorouracil (CMF). Blood hormone levels will be drawn during the first two weeks of each 28-day cycle of chemotherapy for three consecutive cycles and every three months thereafter during CMF administration. Correlations among receptor assay results, endocrine profiles, and tumor recurrences will be sought. (2) Animal *in vivo* studies will use adult female BUF/N rats and NMU-induced mammary tumors. The effects of CMF on tumor, endocrine organs, and fecundity will be studied.

Progress: Six additional Chicago area hospitals are collaborating with the University of Illinois and Cook County Hospitals to provide the required number of patients. The organizational efforts have been completed and patient acquisition to the study began in October 1978. For comparison with the course of the premenopausal study patients, a small group of postmenopausal patients will be treated identically and follow-up will be maintained on a small number of premenopausal patients with Stage I disease who receive no adjuvant chemotherapy.

Project Officers: Mary E. Sears, M.D., Richard Sherins, M.D.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $125,000

CONTRACT RESEARCH SUMMARY

Title: Endocrine Therapy Plus Chemotherapy in Patients with Breast Cancer

Principal Investigator: Dr. David T. Kiang
Performing Organization: University of Minnesota
City and State: Minneapolis, MN

Contract Number: N01-CB-64000
Starting Date: 6/1/76 Expiration Date: 5/31/79

Goal: To study the effect on metastatic breast cancer of combining chemotherapy with estrogen therapy and to assess the value of estrogen receptor assays for treatment selection.

Approach: Patients eligible to enter this study program will be women who are showing the first evidence of metastatic breast cancer. Estrogen receptor (ER) assay results will determine the pair of treatment regimens to be randomly assigned after stratification according to disease-free interval and disease-dominant site. The patients with ER-negative cancer will receive (1) diethylstilbestrol (DES) plus Cytoxan and 5-fluorouracil (CF) or (2) CF alone. Those with ER-positive cancers will receive either (1) DES and CF in combination or (2) DES alone followed by CF with evidence of DES treatment failure or of tumor progression after initial regression. The patients on whom ER assays cannot be obtained will have the same treatment assignments as those with ER-positive tumors.

The incidence and duration of remission and survival data will be analyzed to determine (1) the effect of combining standard hormonal therapy and chemotherapy and (2) the value of ER assay results in predicting the clinical response to therapy.

Progress: Eighty-eight patients with advanced breast cancer have entered the study. Seventy-five of them were evaluated for response to randomized therapy. The overall response rate was 52%. The response rates in each subgroup are listed in the table.

Response	ER (+)		ER (-)		ER (+) or ER (?)	
	DES	DES + CF	CF	DES + CF	DES	DES + CF
CR + PR	7/11	11/14	0/8	5/9	4/12	12/21

Treatment in 52 patients contained CF. Cystitis occurred in 7 patients, leukopenia (<2,000/cmm) in 16, and thrombocytopenia (<75,000/cmm) in 4. Sixty-seven patients had DES, either alone or with CF; 8 complicated with thrombophlebitis and 2 with "stroke," all occurred in patients receiving the combination of DES + CF. Accrual of patients continues.

Project Officer: Mary E. Sears, M.D.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: 0

CONTRACT RESEARCH SUMMARY

Title: Methods To Predict Chemotherapy Sensitivity

Principal Investigator: Dr. John M. Yuhas
Performing Organization: University of New Mexico
City and State: Albuquerque, NM

Contract Number: N01-CB-74203
Starting Date: 8/1/77 Expiration Date: 7/31/80

Goal: To develop a reliable test system for predicting the effectiveness of chemotherapeutic agents in the treatment of individual breast cancers.

Approach: This project will develop a battery of rat breast tumors, with the tumors being grown as s.c. transplants in vivo and as multicellular tumor spheroids (MTS) in vitro. The following characteristics of these two tumor forms will be compared: transplantability in syngeneic rats or anergic mice, morphology, growth rate, growth fraction, cell cycle parameters, karyotype, and hormone sensitivities. The sensitivity of the two forms of tumor will be compared in terms of responsiveness to standard chemotherapeutic agents used for breast cancer. In vivo tumors will be assayed in terms of growth delay following the i.v. administration of the drug. MTS will be exposed either in vitro or in vivo, by placing them in the peritoneal cavity before i.v. drug injection, and harvested afterward for in vitro growth delay analysis. Further, attempts will be made to grow MTS from established human breast cancer lines, and from clinical biopsy material. If successful, similar studies will be undertaken.

Progress: A total of 15 breast cancer cell lines from mice, rats, and man have been tested for their ability to form MTS. All lines derived from solid growths did so, but none of the lines derived from pleural effusions or ascites tumors did so. In the analyses of the 6 rat breast cancer lines, large differences in sensitivity to standard drugs (ADR, VCR, CYC, 5-FU, MTX, and L-PAM) have been established both in vivo and when grown as MTS. The rank ordering of the lines in terms of resistance to a given drug in the two systems correlates, but determination of the relative resistance of a given line to a series of drugs requires additional information on the relative amount of drugs reaching the s.c. site and the peritoneal contents. These studies are in progress using isotopically labeled samples of the drugs being studied. In vivo exposure followed by in vitro analysis of MTS has been studied for both syngeneic rat tumors and for the human tumor lines, with the latter being exposed in the peritoneal cavities of mice and rats for up to 4 hr. Resistance to the various drugs is not correlated, and in many instances is the product of the three-dimensional organization of the MTS. Evolution of hormone independence has been demonstrated in vitro, and preliminary studies suggest that drug resistance can evolve under similar conditions.

Project Officers: Mary E. Sears, M.D., Richard Knazek, M.D.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $110,000

CONTRACT RESEARCH SUMMARY

Title: Methods To Predict Chemotherapy Sensitivity

Principal Investigator: Dr. Russell Hilf
Performing Organization: University of Rochester
City and State: Rochester, NY

Contract Number: NO1-CB-74204
Starting Date: 7/15/77 Expiration Date: 7/14/80

Goal: To develop a reliable test system for predicting the effectiveness of chemotherapeutic agents in the treatment of individual breast cancers.

Approach: This project will perform assays for a battery of enzymes in primary and metastatic breast cancer specimens and build mathematical models of the biochemical profile. The enzymes include lactate dehydrogenase, pyruvate kinase, glucosephosphate isomerase, and isocitrate dehydrogenase. Hormone receptor assays and histological examinations will also be performed. Data concerning the hosts' biological and oncological histories will be acquired, evaluated, and stored. Analyses of the combined data will test the predictiveness of the mathematical model constructed from the biochemical profile.

Progress: Accession of new cases is occurring at the anticipated rate, with data collection and entry into the computer file proceeding satisfactorily. Forms for patient data at initial entry and for follow-up were devised and are in use. More than 220 patients have been entered into our study, based on the criteria for eligibility, and case review is continuing on 300 other patients whose tumors were obtained during the 7 years prior to initiation of this contract. The logistic regression model which uses LDH vs. PK X ICD X GPI shows a correct classification for response or no response of 85% based on 64 cases. The data set is continually increased in number, and subjected to exploratory investigations. Analysis of data from patients on adjuvant chemotherapy has been initiated to seek out those biochemical and clinical parameters that would aid in identifying patients who would benefit or not benefit from this therapeutic regimen; preliminary results based on the logistic model are most encouraging. We have also identified ~80 patients who received no postmastectomy therapy, and we are evaluating the biochemical properties of their lesions along with several clinical parameters as they relate to recurrence of disease.

Project Officers: Mary E. Sears, M.D., E. Brad Thompson, M.D.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $83,700

CONTRACT RESEARCH SUMMARY

Title: Methods To Predict Chemotherapy Sensitivity

Principal Investigator: Dr. Robert B. Livingston
Performing Organization: University of Texas
City and State: San Antonio, TX

Contract Number: NO1-CB-74205
Starting Date: 8/1/77 Expiration Date: 7/31/78

Goal: To develop a reliable test system for predicting the effectiveness of chemotherapeutic agents in the treatment of individual breast cancers.

Approach: In vitro experiments determine the effects of drugs on the thymidine labeling index of the rat mammary tumor 13762. The drugs are added directly in pharmacologic concentrations or indirectly added via the serum of drug-treated animals. The in vitro effects are compared with the in vivo effects on tumor size and on host survival. Cyclophosphamide, fluorouracil, methotrexate, vincristine, phenylalanine mustard, and adriamycin are the drugs used in the test systems individually and in various combinations.

Progress: Period of exposure of tumor cells to control (untreated) and drug-containing (treated) plasma is now limited to 24 hours with resuspension of cells, and continued incubation in RPMI 11630 + FCS. This has eliminated the problem with nonspecific depression of labeling index by control plasma. Ultrasonic exposure for five minutes and addition of dilute trypsin have eliminated the problem with cell aggregation into large clumps, making cytocentrifuge preparations uniformly readable.

Eighteen experiments (3 adriamycin, 3 cyclophosphamide, 3 methotrexate, 1 vincristine, 2 melphalan, 2 fluorouracil, 2 cyclophosphamide-vincristine, and 2 adriamycin-cyclophosphamide) compared day 7 post-treatment reduction in tumor size in vivo with depression of labeling index in vitro at 72 and 97 hours. Overall assessment of accuracy is about 80% with more "false positives" than "false negatives." Modifications to increase specificity are being explored and include 1 hour short-term exposure to drug, adding a 120-hour time point, and repeatedly washing the cells before their resuspension in synthetic medium.

Project Officers: Mary E. Sears, M.D., Richard Knazek, M.D.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $54,100

CONTRACT RESEARCH SUMMARY

Title: Estrophile Binding and Estrophile Proteins in Human Breast Cancer

Principal Investigator: Dr. Federico Welsch
Performing Organization: Worcester Foundation
City and State: Shrewsbury, MA

Contract Number: N01-CB-43867
Starting Date: 10/16/73 Expiration Date: 10/15/78

Goal: To determine whether the levels and properties of estrophilic proteins are related to the responsiveness of cancer to endocrine treatment.

Approach: This is a type I contract in that the contractor carries out determinations of estrophilic receptor protein (ER) levels on 650 breast cancer samples per year. These tissues are submitted by collaborating clinicians whose protocols have been approved by the project officers. The ER assays are performed by both the sucrose gradient and the charcoal methods, and methodology is under constant monitoring. Histological examination by St. Vincent's Hospital has been carried out on each tissue submitted for ER assay. The contractor also performed steroid sulfurylation determinations on tumor samples of sufficient size. Progesterone receptor assays (PgR) were added in 1977.

Progress: The contractor analyzed more than 1,700 specimens received from five institutions. Forty-five percent of the tumors were found to bind >10 femtomoles of estradiol per mg protein, 14% bound from 3 to 10 femtomoles/mg and 41% bound <3 femtomoles/mg. Ninety-one tumors were also analyzed for progesterone receptor and 495 determinations made of sulfurylase activity by the Brooks method and 187 by the Dao method. The data are currently being correlated with the clinical onset and course of the breast cancers, and with histopathologic findings.

ER/PgR Assay Results

	ER+	ER-	ER-low	Total
PgR+	20	3	11	34
PgR-	22	5	30	57
PgR Not Done	739	226	678	1643
Total	781	234	719	1734

Project Officers: Mary E. Sears, M.D., D. Jane Taylor, Ph.D.
Program: Breast Cancer Treatment
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: 0

CANCER BIOLOGY SUPPORT

CONTRACT RESEARCH SUMMARY

Title: Pre-clinical Studies of Chemotherapeutic Studies of Mycosis Fungoides

Principal Investigator: Dr. R. M. Folk
Performing Organization: Battelle Memorial Institute
City and State: Columbus, OH

Contract Number: NO1-CM-43746
Starting Date: 3/1/75 Expiration Date: 9/28/80

Goal: To apply chemotherapeutic agents developed by DCT for topical treatment of mycosis fungoides, a cutaneous lymphoma.

Approach: To increase the knowledge of the pharmacology of chemotherapeutic agents in topical application, investigation of cutaneous irritation, and percutaneous absorption will be carried out.

Progress: Subcontract has been awarded to the University of Mississippi School of Pharmacy. Cutaneous irritation studies have been done. Reports to date indicate that a few chemotherapeutic agents are irritating to rabbit skin after a single application. Initial percutaneous absorption studies have been completed; testing of additional radio-labeled drugs has been completed. No further testing is planned this year.

Project Officer: Dr. Gary Peck
Program: Cancer Biology Support
Technical Review Group: Ad Hoc Committee
FY 79 Funds: $74,644

CONTRACT RESEARCH SUMMARY

Title: Computer Services

Principal Investigator: Mr. Gary Augustson
Performing Organization: Department of the Treasury
City and State: Washington, DC

Contract Number: YO1-CB-90316
Starting Date: 11/15/78 Expiration Date: 9/30/79

Goal: To provide computer facility for mathematical computations related to biological systems modeling carried out in the Laboratory of Theoretical Biology and other groups in DCBD, NCI.

Approach: N/A

Progress: N/A

Project Officer: Dr. Mones Berman
Program: Cancer Biology Support
Technical Review Group: Ad Hoc Committee
FY 79 Funds: $7,000

CONTRACT RESEARCH SUMMARY

Title: Surface Chemical Characterization of a Transformation-sensitive Cell Surface Glycoprotein and Its Interaction

Principal Investigator:	Frank Holly
Substitute Principal Investigator:	Krzysztof Dolowy
Performing Organization:	Eye Research Institute of Retina Foundation
City and State:	Boston, Massachusetts

Contract Number:	NO1-CB-74209		
Starting Date:	9/30/78	Expiration Date:	12/31/78

Goal: To elucidate the mechanism of action of the adhesive, transformation-sensitive glycoprotein fibronectin (known previously as CSP) by surface chemical analysis.

Approach: Cellular fibronectin is an adhesive glycoprotein that is generally decreased after malignant transformation. It is normally self-associated in aggregates and fibrils. Surface chemical analyses will permit highly sensitive investigations of fibronectin in this self-associated form, to complement standard biochemical approaches that require solubilization of fibronectin. Fibronectin is routinely spread as a monomolecular layer at an air-liquid interface in a Langmuir trough, and the film pressure-area isotherm is determined.

Fibronectin's molecular area is found to increase sharply as calcium is bound by EGTA, suggesting that its tertiary structure may be stabilized by this divalent cation. At approximately pH 9 to 10, the molecular area also increases; this expansion coincides with increased solubility of the molecule. A new theory for cell adhesion proposed by Dolowy and Holly has received direct support by studies with charged zinc electrodes that show differences in adhesive behavior of erythrocytes and living chick embryo fibroblasts as a function of surface potential; the currently dominant DVLO theory cannot explain these differences.

This contract has provided useful, new biophysical information on the surface chemistry of fibronectin and on the relationship of surface potential to normal fibroblast adhesion.

Project Officer: Dr. Kenneth Yamada
Program: Cancer Biology Support
Relevance Review Group: DCBD Board of Scientific Counselors
FY 79 Funds: $8,504

CONTRACT RESEARCH SUMMARY

Title: A Screening System for Topical Chemotherapy of Mycosis Fungoides

Principal Investigator: Dr. Stanford Lamberg
Performing Organization: Johns Hopkins University
City and State: Baltimore, MD

Contract Number: NO1-CB-63927
Starting Date: 6/30/76 Expiration Date: 6/29/80

Goal: To apply chemotherapeutic agents developed by DCT for topical treatment of mycosis fungoides, a cutaneous lymphoma.

Approach: Chemotherapeutic agents are obtained commercially and from the DCT, NCI, and these agents are being screened by clinical patch testing in patients with mycosis fungoides.

Progress: Investigative new drug applications have been obtained from the FDA and patch test screening of these agents has begun at Hopkins and other affiliated institutions comprising the Mycosis Fungoides National Cooperative Group. More widespread testing of agents found to be active by patch test screening will begin shortly.

Project Officer: Dr. Gary Peck
Program: Cancer Biology Support
Technical Review Group: Ad Hoc Committee
FY 79 Funds: $66,791

CONTRACT RESEARCH SUMMARY

Title: Radioimmunoassay of Immunoglobulin Molecules

Principal Investigator: Dr. James Harness
Performing Organization: Meloy Laboratories, Inc.
City and State: Springfield, Virginia

Contract Number: NO1-CB-63932
Starting Date: 3/15/79 Expiration Date: 3/14/80

Goal: To perform radioimmunoassays of immunoglobulin molecules IgM, IgA, IgG, IgD, and IgE in lymphocyte culture supernatants or in other biological fluids.

Approach: The contractor is to perform determinations of human IgG, IgA, IgM, IgD, and IgE, and mouse IgA and IgM by double antibody radioimmunoassay by procedures defined by the project officer and using reagents supplied by the project officer. This contract provides critically required research support for studies on the nature of immunodeficiencies that are associated with a high incidence of malignancy and on the cause of the immunodeficiency associated with malignancies of the B cell or plasma cell system. In addition, these studies are directed at defining retained immunological capabilities of T-cell leukemias.

Progress: The contract has established radioimmunoassays for IgG, IgA, IgM, and IgE of man and for IgA and IgM of the mouse. These assays have been used to quantitate the rate of immunoglobulin synthesis by pokeweed mitogen-stimulated, peripheral blood lymphocytes of man or of splenic lymphocytes of the mouse in in vitro cultures. Patients with a T-cell leukemia associated with the Sezary syndrome have been shown to have a malignant expansion of helper T cells whereas a patient with acute lymphocytic leukemia was shown to have a malignancy of the suppressor T cells. A subset of patients with common variable hypogammaglobulinemia has been shown to have excessive numbers of suppressor T cells that inhibit gammaglobulin synthesis by B cells. Certain patients with post marrow transplant immunodeficiency have deficient B-cell function whereas others have excessive suppressor T-cell activity. Patients with multiple myeloma have hypogammaglobulinemia due in part to excessive numbers of suppressor macrophages. These studies are defining the nature of disorders of the immune system that led to a high incidence of cancer. In addition, they provide information on the cause of immunodeficiency that arises secondary to certain forms of malignancy. Finally, these studies have provided insights into the retained functions of T-cell leukemias.

Significance to Cancer Research: These studies will help elucidate the status of the immune system during development of cancer.

Project Officer: Thomas A. Waldmann, M.D.
Program: Cancer Biology Support
Technical Review Group: Ad Hoc Review Group
FY 79 Funds: $142,665

CONTRACT RESEARCH SUMMARY

Title: Computer Services

Principal Investigator:	Mr. Martin Shaver
Performing Organization:	Computer Services Division National Bureau of Standards
City and State:	Gaithersburg, MD

Contract Number: Y01-CB-80047
Starting Date: 7/1/73 Expiration Date: 9/30/79

Goal: To provide computer facility for mathematical computation related to biological systems modeling carried out in the Laboratory of Theoretical Biology and other groups in DCBD, NCI.

Approach: N/A

Progress: N/A

Project Officer: Dr. Mones Berman
Program: Cancer Biology Support
Technical Review Group: Ad Hoc Committee
FY 79 Funds: $55,000

CONTRACT RESEARCH SUMMARY

Title: Small Animal Holding Facility

Principal Investigator: Andrew S. Tegeris
Performing Organization: Pharmacopathics Research Laboratories
City and State: Laurel, MD

Contract Number: N01-CB-33887
Starting Date: 7/1/73 Expiration Date: 12/30/78

Goal: To maintain and observe animals on experiments designed to develop the means to reduce the effectiveness of external agents for producing cancer with a variety of types of materials, particularly mineral fibers and tobacco products.

Approach: Improve animal models of human cancer and evaluate effects of combined factors by assessing the response of the pleura of the rat to fibers and other forms of minerals that could be expected to reach this site in man.

Progress: We are currently testing carcinogenic response to many types of durable fibers in the pleura of the rat. Results suggest that fiber size is critical to carcinogenesis by asbestos and other fibers. Tobacco smoke fractions and air pollutant fractions are being evaluated for carcinogenicity on a quantitative level.

Significance to Cancer Research: Animal models of human cancer are essential to identifying causative agents, mechanisms of action, and therapeutic trials.

Project Officer: Mearl F. Stanton, M.D.
Program: Cancer Biology Support
Technical Review Group: Ad Hoc Relevance Review Group
FY 79 Funds: $100,000 (6 months)

CONTRACT RESEARCH SUMMARY

Title: A Study of Phylogenetic Aspects of Neoplasia

Principal Investigator: Dr. John C. Harshbarger
Performing Organization: Smithsonian Institution
City and State: Washington, D.C.

Contract Number: NO1-CB-33874
Starting Date: 7/1/73 Expiration Date: 6/30/80

Goal: To collect, examine, classify, and preserve neoplasms in cold-blooded vertebrate and invertebrate animals, and to study experimentally the development of tumors in lower animals.

Approach: The principal investigator directs the operation of a registry of tumors in lower animals. Specimens are acquired from personal field investigations or through submittal by other investigators. The specimens are examined grossly, histologically, and in some cases by electron microscopy. Diagnoses are established and the specimens are described. The world literature on tumors in lower animals is collected. Field investigations and experimental inductions of tumors in lower animals are carried out. Publications of the findings are made. The Registry also serves in a consulting capacity to other agencies concerned with diseases in lower animals, such as the Environmental Protection Agency, the U.S. Bureau of Fisheries (Commerce Dept.), and the Environmental Health Center.

Progress: Specimen accessions during the past year numbered 203, bringing the total collection to 2,040. Fifty-two percent of the new accessions are neoplasms, the majority from mollusks, teleost fish, amphibians, and reptiles. While lesions that appear to be neoplastic by morphological criteria continue to be found in mollusks and arthropods (insects alone represented), the other invertebrate phyla appear to have no representation except for lesions of controversial nature. Types of tumors accessioned are listed in the 1977 Progress Report of the Registry. Particularly significant were hepatocellular neoplasms in tomcod from the lower Hudson River and in flounder from Pacific Northwest coastal waters, suggesting the presence of hepatic carcinogens in the environments in these areas. A group of spontaneous growths in the skin of axolotls in a laboratory colony were identified by electron microscopy as mast cell tumors, the first tumors of this cell type to be recognized in non-mammals. Foreign contributions came from Canada, England, New Zealand, The Netherlands, Germany, Japan, and USSR. Three original reports were published by the PI during the 1978 fiscal year.

Significance to Cancer Research: Field studies and anatomical studies indicate environmental carcinogens that may be of importance in human cancer epidemiology or may be useful in designing analytical experiments to determine mechanisms of tumorigenesis. The continued apparent paucity of neoplasms among invertebrates other than insects and mollusks points to a need for basic research in this area.

Project Officer: Clyde J. Dawe, M.D.
Program: Cancer Biology Support
Technical Review Group: Ad Hoc Committee
FY 79 Funds: $150,000

CONTRACT RESEARCH SUMMARY

Title: Preparation and Analysis of Cell Surface Protein (CSP) Fractions

Principal Investigator: Dr. David Schlesinger
Performing Organization: University of Illinois
City and State: Chicago, IL

Contract Number: NO1-CB-74214
Starting Date: 9/30/78 Expiration Date: 9/30/79

Goal: To prepare and analyze the structure of cell surface protein.

Approach: Two methods have been used to prepare the C-terminal fragment of cell surface protein after pepsin cleavage of the molecule. One method employs two-dimensional electrophoresis, the second is a column method. The material obtained from the C-terminus is now being sequenced. A small amount of sequence information is available. This sequence is important because it will be used to authenticate the identification of a C-DNA clone isolated by John Fagan which has been tentatively identified as coming from the fibronectin gene.

Another fragment of CSP has been prepared which contains the collagen binding site. The strucutre of this fragment is currently under investigation.

CSP has been shown to be absent in many kinds of cancer cells and its absence has been correlated with the tumorigenicity of some cells. The knowledge of the structure of CSP will further the understanding of the cancer process. Dr. Schlesinger will continue to characterize and obtain the structure of various fragments of CSP as described above.

Project Officer: Dr. Ira Pastan
Program: Cancer Biology Support
Technical Review Group: Ad Hoc Committee
FY 79 Funds: $46,000

CONTRACT RESEARCH SUMMARY

Title: Computer Services

Principal Investigator: Mr. Wayne H. Anderson
Performing Organization: USAARDC
City and State: Aberdeen Proving Ground, MD

Contract Number: Y01-CB-80315
Starting Date: 5/1/78 Expiration Date: 9/30/79

Goal: To provide computer facility for mathematical computations related to biological systems modeling carried out in the Laboratory of Theoretical Biology and other groups in DCBD, NCI.

Approach: N/A

Progress: N/A

Project Officer: Dr. Mones Berman
Program: Cancer Biology Support
Technical Review Group: Ad Hoc Committee
FY 79 Funds: $5,000

CONTRACT RESEARCH SUMMARY

Title: Preparation of Purified Wheat Proteins and Wheat Protein Fractions

Principal Investigator: Dr. Donald Kasarda
Performing Organization: U.S. Department of Agriculture
City and State: Berkeley, CA

Contract Number: YO1-CB-60312
Starting Date: 10/1/78 Expiration Date: 9/30/79

Goal: To obtain chemically defined fraction of wheat gliadin for use in studies of gluten-sensitive enteropathy (coeliac sprue).

Approach: Wheat gluten will be chemically fractionated and subjected to cyanogen bromide cleavabe. Homogeneous fragments of gliadin, as well as gliadin itself, will then be supplied.

Progress: The contractor has supplied a-gliadin according to the contract schedule. Using these materials as toxic component of wheat has been tentatively identified with the help of organ culture technology. In addition, the a-gliadin has been used in in vitro studies of responses of lymphoid cells to defined gliadin preparations - cells obtained from both patients and normals. Finally, a-gliadin has been used in a radiometric assay of anti-bliadin production by lymphoid cells in vitro which will ultimately enable us to study the regulation of anti-glidin responses in vitro.

Significance to Cancer Research: Gluten-sensitive enteropathy is a disease associated with a high incidence of malignancy. Elucidation of the pathogenesis of gluten-sensitive enteropathy will provide insight into the factors which lead to the onset of malignant disease.

Project Officer: Dr. Warren Strober
Program: Cancer Biology Support
Technical Review Group: Breast Cancer Task Force Committee
FY 79 Funds: $18,600

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